KemmelCal Inc.

Attention: Dr. Susan Carlson
Office of Food Additive Safety (HFS-200)
Center for Food Safety & Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
5001 Campus Drive
College Park, MD 20740

Re: GRAS Notification *-Ashitaba Cha/cone Powder {8%}* **June 25, 2021**

Dear Dr. Carlson:

KemmelCal Inc., acting as Agent for Japan Bio Science Laboratory-USA, Incorporated ("JBSL-USA") and in accordance with 21 CFR Part 170 Subpart E, is submitting for FDA review Form 3667 and the enclosed CD containing a GRAS Notice for *Ashitaba Cha/cone Powder {8%).* **JBSL-USA has determined that** *Ashitaba Cha/cone Powder {8%)* **under the conditions of its intended use in foods is GRAS based on scientific procedures, and is therefore exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act.**

The electronic files contained on the CD were scanned for viruses and were found to be virus-free by McAfee Total Protection prior to submission.

Should any questions or concerns arise with regard to this GRAS Notice, please feel free to contact me directly.

Sincerely,

Katrina Emmel, Ph.D.
(Agent of the Notifier)
President
KemmelCal Inc.
947 Martina Circle
Corona, CA 92879

Email: katrina.emmel@kemmelcal.com
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Enclosure: GRAS Notice for JBSL-USA *-Ashitaba Cha/cone Powder {8%}*

GRAS Notice for Ashitaba Chalcone Powder (8%)

Prepared by

Japan Bio Science Laboratory

Walnut Creek, CA

June 25, 2021

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Part 1. Signed Statements and Certification

1.1 Basis of GRAS Status Claim

In accordance with the Section 201(s) of the Food, Drug, and Cosmetic Act (FD&C Act), and pursuant to 21 CFR §170 Subpart E, Japan Bio Science Laboratory-USA, Incorporated (hereinafter "JBSL-USA") has concluded that Ashitaba Chalcone Powder (8%), manufactured according to Current Good Manufacturing Practices (CGMP), and which meets the specifications described in Part 2 of this dossier, is Generally Recognized as Safe (GRAS) under the conditions of its intended use.

JBSL-USA's GRAS conclusion is based on scientific procedures. A literature search was conducted through May 17, 2021 to identify any scientific or regulatory publications regarding the safety and toxicity of Ashitaba Chalcone Powder (8%), including any adverse reports. The references that were deemed relevant to this safety review are listed in Part 7. The combination of compositional details, detailed manufacturing process description, dietary exposure estimate, and safety and toxicity data provide the scientific procedure basis for this GRAS conclusion. No confidential, non-public, or generally inaccessible information was used by JBSL-USA to reach this GRAS conclusion.

1.2 Name and Address of Notifier

Vincent Hackel

President/CEO

JBSL-USA

1547 Palos Verdes Mall, #131

Walnut Creek, CA 94597

1.3 Common Name of Notified Substances

The notified substance is Ashitaba Chalcone Powder (8%). The common or usual names that may be used to described the preparations are ashitaba, asitaba, *Angelica keiskei*, ashitaba chalcone sap powder, and ChalCurb-P8.

1.4 Intended Conditions of Use in Foods

Ashitaba Chalcone Powder (8%) is intended to be used as an ingredient in conventional foods as described further in Part 3 of this dossier. JBSL-USA's Ashitaba Chalcone Powder (8%) preparation is not intended to be used as a color additive; therefore, it is exempt from the definition of a color additive under section 201(t) of the FD&C Act and FDA's implementing regulations in 21 CFR §7.30(f) and (g).

1.5 Basis for the GRAS Conclusion

JBSL-USA's GRAS conclusion is based upon scientific procedures, as discussed in detail in this dossier, in accordance with 21 CFR §170.30(a) and (b).

1.6 Exclusion from Premarket Approval Requirements

Ashitaba Chalcone Powder (8%) is not subject to premarket approval requirements of the Food, Drug, and Cosmetic Act (FD&C Act) based on JBSL-USA's conclusion that Ashitaba Chalcone Powder (8%) is GRAS under the intended conditions of use.

1.7 Availability of Information

The composite data and information that serve as the basis of this GRAS conclusion will be available to the Food and Drug Administration (FDA) during customary business hours at JBSL-USA's facility located at 1547 Palos Verdes Mall, #131, Walnut Creek, CA, 94597.

1.8 Exemption from Freedom of Information Act (FOIA) Disclosure

JBSL-USA certifies that no data or information contained herein are exempt from disclosure under the Freedom of Information Act (FOIA).

1.9 Food Safety and Inspection Service (FSIS) Statement

JBSL-USA does not intend to add Ashitaba Chalcone Powder (8%) to foods that come under United States Department of Agriculture (USDA) jurisdiction, such as meat or poultry products; therefore, sharing the information contained herein with the Food Safety and Inspection Service (FSIS), trade secret or otherwise, does not apply.

1.10 Certification

JBSL-USA certifies, to the best of our knowledge, that this GRAS dossier is a complete, representative, and balanced assessment that includes all information, both favorable and unfavorable, known to JBSL-USA and relevant to the evaluation of safety and GRAS status of Ashitaba Chalcone Powder (8%).

Signed,

Katrina V. Emmel, Ph.D.

Agent for JBSL-USA

KemmelCal Inc.

947 Martina Circle

Corona, CA 92879

Date: June 25, 2021

Part 2. Identity, Method of Manufacture, Specifications, and Technical Effect of the Notified Substances

2.1 Biological Information

Ashitaba Chalcone Powder (8%) is prepared from the sap of *Angelica keiskei* (Miq.) Koidz. (syn. *Archangelica keikei* Miq.), which was first identified in *Florae Symbolae Orientali-Asiaticae* in 1930 (Kew Royal Botanic Gardens, 2019). *A. keiskei* is a perennial herb in the carrot family native to Japan and cultivated in areas of Eastern Asia, including Japan and Korea. Ashitaba originated in Hachijo-shima, but is also grown in the Izu Peninsula, Miura Peninsula, Izu Islands, and Ohshima. It is often referred to as "ashitaba" or "asitaba" in Japan and "sinsuncho" in Korea (Fukuo et al., 2005). A photograph of the aerial portion of *A. keiskei* is shown in Figure 1.

***Figure 1. Angelica keiskei* Plant**

The taxonomic classification[1] of Ashitaba is as follows:

Kingdom	Plantae
Order	Apiales
Family	Apiaceae (Umbelliferae)
Subfamily	Apioideae
Tribe	Selineae
Genus	*Angelica*
Species	*Angelica keiskei*

2.2 Ashitaba Sap

Multiple studies have characterized the components of various portions of *A. keiskei*. Ashitaba leaves, stems, and roots contain noteworthy amounts of vitamin C, vitamin A, vitamin K, and dietary fiber, as well as chalcones, flavanones, coumarins, and carotenoids (Ohkura et al., 2018; Kim et al., 1992; Xie et al., 2017).

Juice extracted from ashitaba stems has a high water content. Ashitaba yellow sap, shown in Figure 2, is an exudate that contains chalcones, flavanones, and coumarins (Akihisa et al., 2003; Ohnogi et al., 2012e).

***Figure 2. Angelica keiskei* Yellow Sap**

[1] Previously obtained on USDA's Germplasm Resources Information Network: Taxon *Angelica keiskei* (Miq.) Koidz

Kim et al. (1992) analyzed the nutritional component composition of the juice obtained from the stem and whole aerial plant portion of *A. keiskei.* A summary of their findings is provided in Table 1.

Table 1. Proximate Analysis of* Angelica keiskei *Koidz Stem Juice and Aerial Whole Plant[a]

Analyte	Stem Juice	Aerial Whole Plant[b]
Proximate Analysis (g/100 g wet weight)		
Moisture	95.06	88.75
Lipids	0.92	2.75
Protein	1.28	2.53
Ash	1.00	2.00
Total Sugar	3.85	2.98
Reducing Sugar	2.63	2.27
Fiber	1.69	7.73
Minerals (mg/100 g wet weight)		
Calcium	109.00	156.24
Copper	0.02	0.05
Iron	0.38	1.63
Potassium	141.60	209.20
Magnesium	9.52	13.60
Manganese	0.06	0.72
Sodium	30.46	52.40
Potassium	22.60	31.04
Zinc	0.11	0.48
Germanium	0.04	0.03
Selenium	Not detected	Not detected
Cobalt	Not detected	Not detected
Vitamins (mg/100 g wet weight)		
Ascorbic Acid	9.40	20.20

Analyte	Stem Juice	Aerial Whole Plant[b]
β-Carotene	0.09	0.51
Thiamin	0.19	2.30
Riboflavin	2.29	1.07
Total Amino Acids (mg/ 100 g wet weight)		
Lysine	Trace	92.40
Histidine	8.80	79.40
Arginine	9.30	133.50
Aspartic acid	16.00	182.10
Threonine	10.60	138.60
Serine	8.30	123.90
Proline	Trace	Trace
Glutamic acid	26.70	280.40
Glycine	26.70	280.20
Alanine	17.20	194.20
Valine	12.60	102.20
Methionine	3.10	26.90
Isoleucine	7.60	74.00
Leucine	14.80	171.60
Tyrosine	1.90	76.60
Phenylalanine	9.30	105.70
Tryptophan	0.50	Trace
Total amino acids	181.40	1,989.70
Total essential amino acids	67.30	790.80
Fatty Acid Composition of Lipids (%)		
Caprylic	1.57	0.31
Capric	Trace	0.37
Lauric	Trace	0.93

Analyte	Stem Juice	Aerial Whole Plant[b]
Tridecanoic	Trace	0.93
Myristic	0.66	1.07
Pentadecanoic	Trace	Trace
Palmitic	22.80	15.79
Palmitoleic	2.55	4.97
Margaric	1.09	6.93
Stearic	2.79	1.35
Oleic	3.99	1.80
Linoleic	43.29	31.14
Linolenic	11.35	27.44
Arachidic	Trace	0.81
Unknown	9.91	6.28
Total saturated fatty acid	38.82	34.87
Total unsaturated fatty acid	61.18	65.35

[a] Adapted from Kim et al. (1992)

[b] The whole aerial portions of the plant except for the root

JBSL-USA's ashitaba yellow sap is composed largely of moisture, fat, carbohydrates, and protein, with minor components including minerals, carotenoids, and flavonoids, including chalcones and coumarins. Table 2 provides a summary of the typical chemical composition of JBSL-USA's ashitaba yellow sap.

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Table 2. Typical Composition of JBSL-USA's* A. keiskei *Yellow Sap

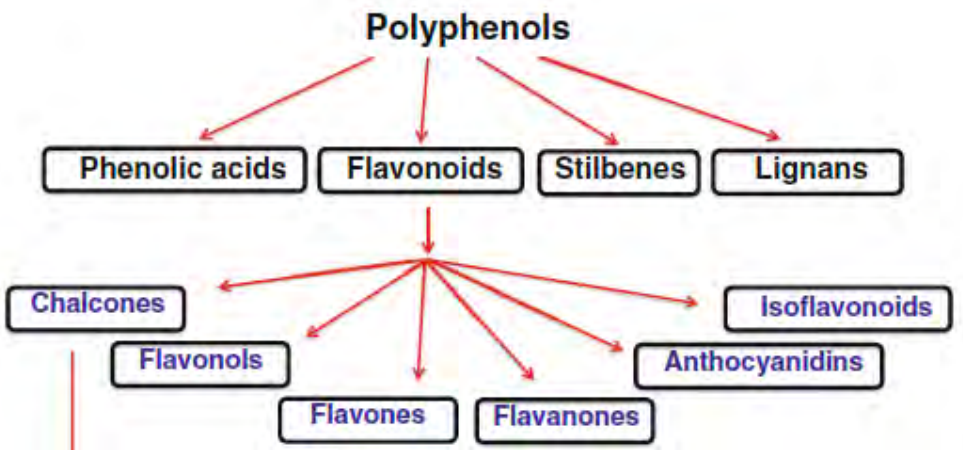
Component	Typical Content
Moisture	71.9%
Ash	0.2%
Protein (Kjeldahl)	0.8%
Fat	18.7%
Carbohydrate	8.4%
Energy	205 kcal/100 g
Total Chalcones[a]	9.08%

[a] As the sum of xanthoangelol and 4-hydroxyderricin

2.2.1 Flavonoids

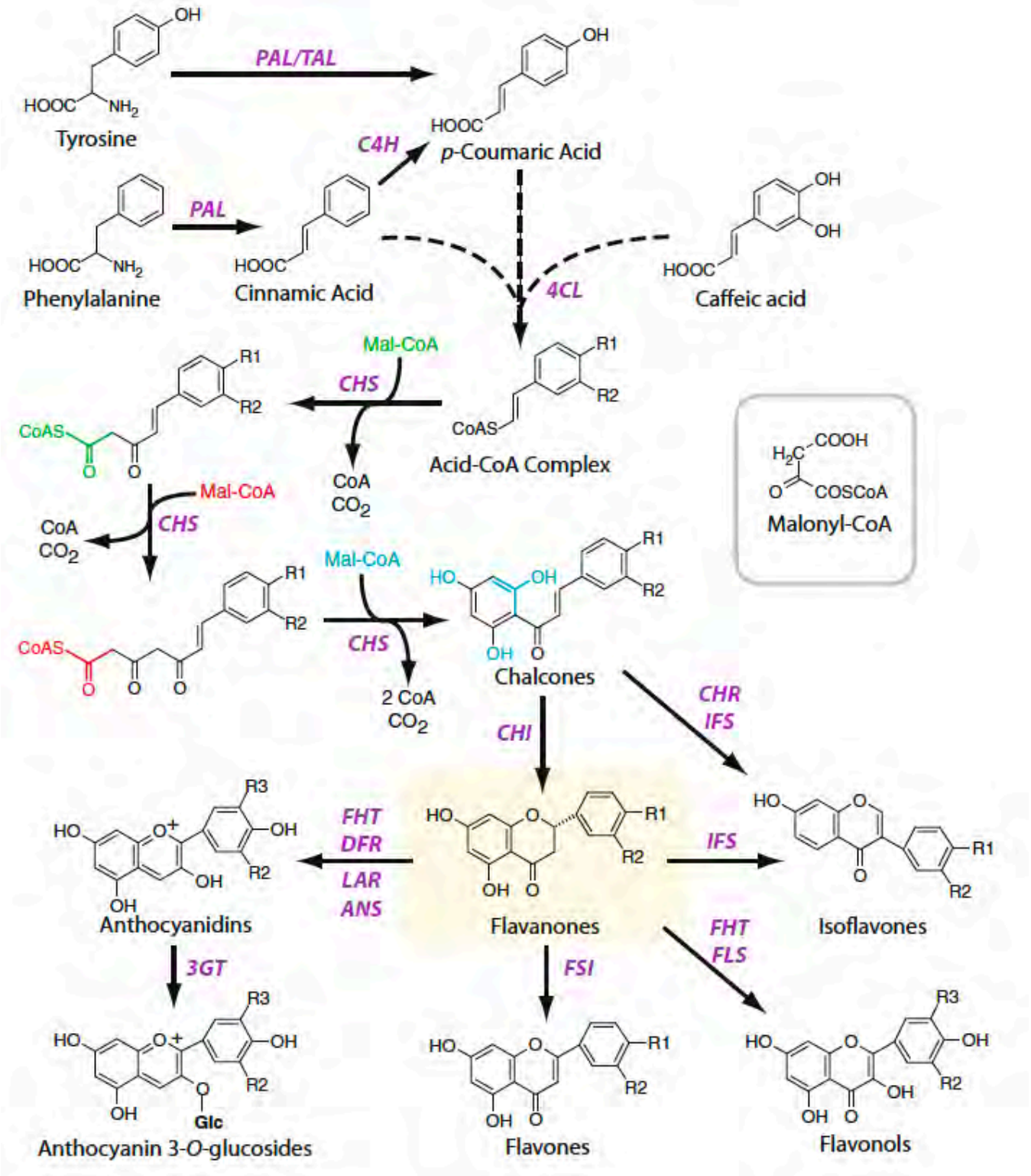
As one of the main polyphenol family members, flavonoids are a diverse class of ubiquitous secondary metabolites found in plants (Fowler and Koffas, 2009; Orlikova et al., 2011; Panche et al., 2016). Flavonoids can be divided into a number of subclasses including chalcones, flavonols, flavones, flavanones, anthocyanidins, and isoflavonoids, as depicted in Figure 3. As shown in the biosynthetic pathway provided in Figure 4, flavonoids are synthesized in a complex pathway that begins with the amino acids tyrosine and phenylalanine.

***Figure 3. Diagram of the Polyphenols*[a]**



[a] From Orlikova et al. (2011)

Figure 4. Botanical Biosynthesis Pathway for Flavonoids[a]



[a] From Fowler and Koffas (2009)

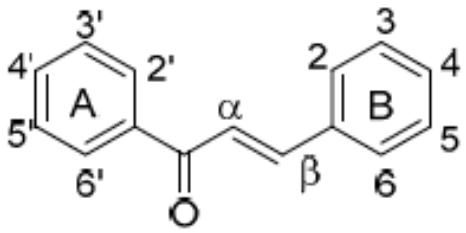
Botanicals, including fruits and vegetables, contain flavonoids in the form of flavonols, flavones, and flavanones. The dietary intake of flavonoids was estimated to range between 20 mg per day (in the United States, Denmark, and Finland) to > 70 mg per day (in Holland) (Beecher, 2003). However, more recent publications have reported much higher total flavonoid intakes in US and European populations. Using National Health and Nutrition Examination Survey (NHANES) food consumption data for adults aged 19 years and older, it was estimated that dietary intake of flavonoids in the US was 201.9 mg per day (NHANES 1999-2002) and 200.1 mg per day (NHANES 2007-1010) (Kim et al., 2016b). Using food consumption data compiled by the European Food Safety Authority (EFSA) and the FLAVIOLA Food Composition Database, the mean intake of flavonoids in Europe was estimated to be 428 ± 49 mg per day (Vogiatzoglou et al., 2015).

Total intake of flavonol and flavone (two subfamilies of flavonoids) is estimated to range between 3 and 65 mg per day, where Finland has the lowest reported intake at 3 mg per day and Japan has the highest intake reported at 65 mg per day (Justesen et al., 2000).

2.2.1.1 Chalcones

Chalcones are open-chain flavonoids with an α, β-unsaturated ketone moiety. They are colored compounds with a general (*E*)-1,3-diphenylpropen-1-one structure (Rosa et al., 2019), the general structure of which is shown in Figure 5. The term "chalcone" is derived from the Greek "*chalcos,"* which means bronze, which is the color of most natural chalcones (Zhuang et al., 2017). Carthamin, a red pigment isolated from safflower (*Carthamus tinctorius*), was the first known naturally-occurring chalcone reported by Kametaka and Perkin in 1910 (Bohm and Stuessy, 2001). Chalcones are plentiful in edible vegetables, fruits, tea, soy-based products, safflower, licorice, potatoes, and spices (Díaz-Tielas et al., 2016; Orlikova et al., 2011).

Figure 5. General Chalcone Structure[a]

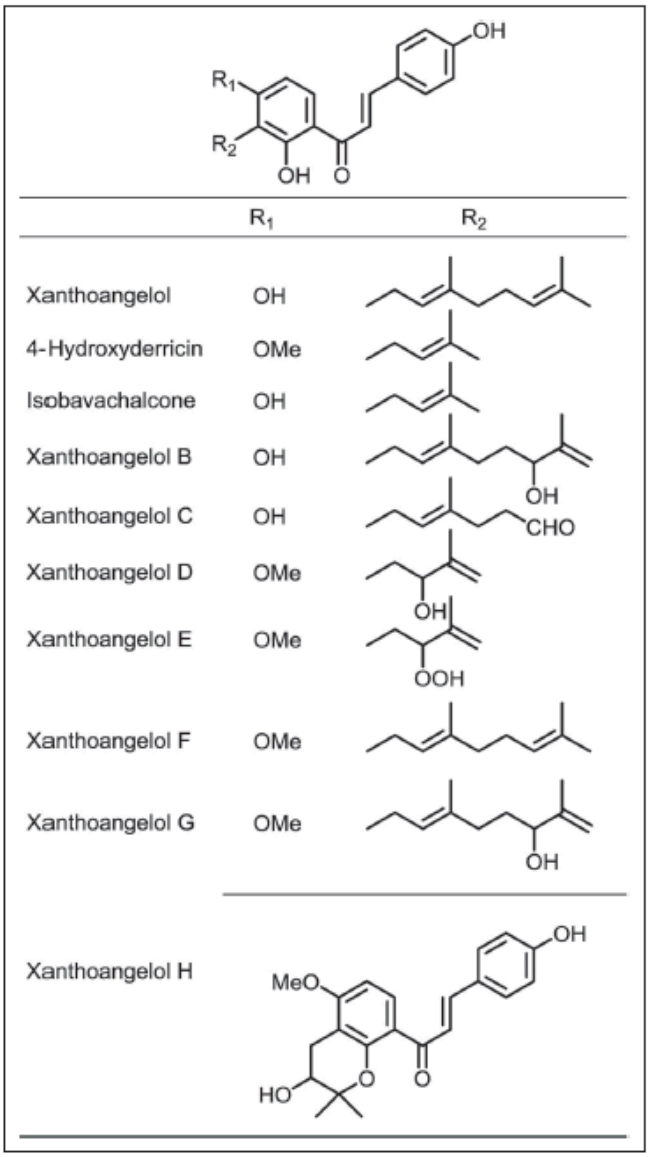


[a] From (Rosa et al., 2019)

Kim et al. (2014) reported that the concentration of chalcones varies between the different portions of the plant, as follows: root bark (10.51 mg per g) > stems (8.52 mg per g) > leaves (2.63 mg per g) > root cores (1.44 mg per g). Furthermore, Nagata et al. (2007) estimated that there are about 200 to 300 mg chalcones per 100 grams of edible raw ashitaba leaf.

JBSL-USA estimates that ashitaba yellow sap contains over 20 chalcones. The most prevalent chalcones are xanthoangelol (XA) and 4-hydroxyderricin (4-HD), which account for more than 90% of the total chalcones identified in ashitaba. Minor components include the xanthoangelols B, C, D, E, F, G, and H, as well as isobavachalcone, 4,2′-4′-trihydroxy-3′-[(2*E*,5*E*)-7-methoxy-3,7-dimethyl-2,5-octadienyl]chalcone, (±)-4,2′,4′-trihydroxy-3′-[(2*E*)-6-hydroxy-7-methoxy-3,7-dimethyl-2-octenyl]chalcone, 4,2′,4′-trihydroxy-3′-[(2*E*)-3-methyl-5-(1,3-dioxolan-2-yl)-2-pentenyl]chalcone, 2′,3′-furano-4-hydroxy-4′-methoxychalcone, and (±)-4-hydroxy-2′-3′-(2,3-dihydro-2-methoxyfurano)-4′-methoxychalcone (Ohkura et al., 2018; Kil et al., 2017). The structures of many of the chalcones identified in ashitaba are shown in Figure 6.

Figure 6. Ashitaba Chalcones[a]



[a] From Ohkura et al. (2018)

A summary of the chalcones identified in ashitaba stems or yellow sap reported in the published scientific literature to date is provided in Table 3.

Table 3. Chalcones Extracted from Ashitaba Yellow Sap or Stem

Chalcone	Reference
Deoxydihydroxanthoangelol H	Akihisa et al. (2006)
Deoxyxanthoangelol H	Akihisa et al. (2006)
Dorsmannin A	Akihisa et al. (2006)
4-Hydroxyderricin	Akihisa et al. (2003)
Isobavachalcone	Akihisa et al. (2003)
Xanthoangelol	Akihisa et al. (2003)
Xanthoangelol B	Aoki et al. (2008)
Xanthoangelol C	Baba et al. (1998)
Xanthoangelol D	Ohkura et al. (2011)
Xanthoangelol E	Fujita et al. (1992)
Xanthoangelol F	Akihisa et al. (2003)
Xanthoangelol H	Akihisa et al. (2003)
Xanthoangelol I	Akihisa et al. (2006)
Xanthoangelol J	Akihisa et al. (2006)
Xanthokeismin A	Aoki et al. (2008)
Xanthokeismin B	Aoki et al. (2008)
Xanthokeismin C	Aoki et al. (2008)

Kim et al. (2014) quantified a number of chalcones identified in a methanolic extract of dried ashitaba stems, as summarized in Table 4.

***Table 4. Chalcone Content of* Angelica keiskei *Stems*[a]**

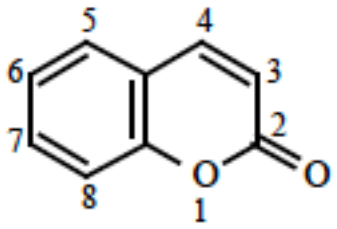
Chalcone	Concentration (mg/g dry weight)
Xanthoangelol	5.05 ± 0.18
4-Hydroxyderricin	1.97 ± 0.12
Xanthoangelol F	1.02 ± 0.09
Isobavachalcone	0.37 ± 0.01
Xanthoangelol B	0.11 ± 0.00

[a] Adapted from Kim et al. (2014)

2.2.1.2 Coumarins

Coumarins are a chemical class of benzo-α-pyrones that are commonly found in edible vegetables, fruits, seeds, nuts, coffee, tea, and wine. Coumarin was first isolated in 1820 from 'Coumarou,' also referred to as the tonka bean (*Dipteryx odorata* Willd., Fabaceae). Coumarins are plentiful in certain essential oils, at levels ranging from 7,000 parts per million (ppm) in cinnamon bark oil up to 87,300 ppm in cassia leaf oil. Coumarins often occur in higher plants, with the highest concentrations observed in the fruits, followed by the roots, stems, and leaves (Lacy and O'Kennedy, 2004). The general coumarin structure is shown in Figure 7.

***Figure 7. General Coumarin Structure*[a]**



[a] From Lacy and O'Kennedy (2004)

A summary of the coumarins identified in ashitaba stems or yellow sap reported in the published scientific literature to date is provided in Table 5.

Table 5. Coumarins Extracted from Ashitaba Yellow Sap or Stem

Coumarin	Reference
(8*S*,9*R*)-8-angeloxy-8,9-hihyrooroselol	Shibano et al. (2009)
(3'R)-3'-hydroxycolumbianidin	Akihisa et al. (2003)
Isolaserpitin	Akihisa et al. (2003)
Isopimpinellin	Akihisa et al. (2006)
Laserpitin	Akihisa et al. (2003)
Osthenol	Akihisa et al. (2006)
Pteryxin	Akihisa et al. (2003)
Selinidin	Akihisa et al. (2003)
3'-Senecioyl khellactone	Akihisa et al. (2003)
4'-Senecioyl khellactone	Akihisa et al. (2003)
Xanthotoxin	Akihisa et al. (2006)

It has been reported that the composition and concentration of coumarins in *A. keiskei* yellow sap varies based on geographical location. The (8*S*-9*R*)-8-angeloxy-8,9-dihyrooroselol (DHO), laserpitin (LAS), and isolaserpitin (ILA) content of yellow sap derived from *A. keiskei* harvested in Oshima and Hachijo was reported by Shibano et al. (2009) and results are summarized in Table 6.

Table 6. Variation of Coumarins Extracted from* A. keiskei *Yellow Sap[a]

Strain	DHO (%)	LAS (%)	ILA (%)
Oshima-1 (n=3)	0.210±0.008	ND	ND
Oshima-2 (n=3)	0.092±0.002	ND	ND
Oshima-3 (n=3)	0.172±0.012	ND	ND
Oshima-4 (n=3)	0.259±0.010	ND	ND
Oshima-5 (n=3)	0.130±0.016	ND	ND
Oshima-6 (n=3)	0.166±0.013	ND	ND
Oshima-7 (n=3)	0.198±0.004	ND	ND

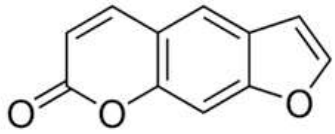
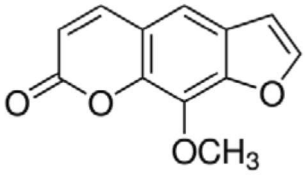
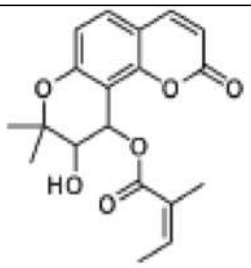
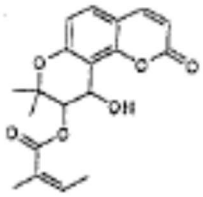
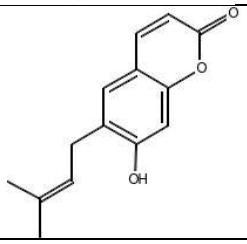
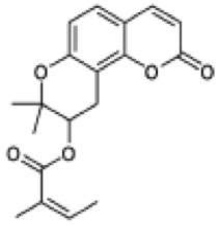
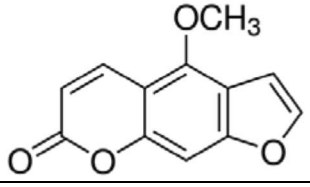
Strain	DHO (%)	LAS (%)	ILA (%)
Oshima-8 (n=3)	0.076±0.001	ND	ND
Oshima-9 (n=3)	0.099±0.004	ND	ND
Oshima-10 (n=3)	0.279±0.024	ND	ND
Oshima-11 (n=3)	0.090±0.006	ND	ND
Oshima-12 (n=3)	0.064±0.001	ND	ND
Oshima-13 (n=3)	0.250±0.020	ND	ND
Oshima-14 (n=3)	0.127±0.003	ND	ND
Oshima-15 (n=3)	0.143±0.044	ND	ND
Oshima-16 (n=3)	0.233±0.003	ND	ND
Oshima-17 (n=3)	0.097±0.007	ND	ND
Oshima-18 (n=3)	0.103±0.003	ND	ND
Hachijo-1 (n=2)	ND	0.092±0.007	0.068±0.003
Hachijo-2 (n=2)	ND	0.066±0.001	0.040±0.003

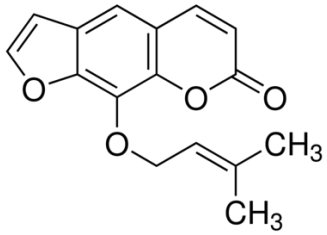
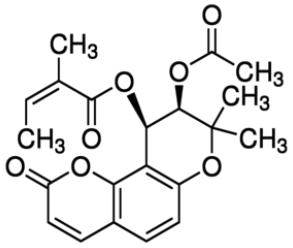
[a] Adapted from Shibano et al. (2009)
DHO – (8*S*,9*R*)-8-angeloxy-8,9-dihyrooroselol; ILA – isolaserpitin; LAS – laserpitin; ND – not detected

Kim et al. (2014) isolated a number of coumarins from a methanolic extract of dried ashitaba stems, as summarized in Table 7.

[Remainder of page is blank]

***Table 7. Coumarins Isolated from* Angelica keiskei *Stems*[a]**

Coumarin	Concentration (mg/g dry weight)[a]	Structure
Psoralen[b]	0.56± 0.05	
Xanthotoxin[c]	1.23 ± 0.06	
Laserpitin[d]	1.03 ± 0.06	
Isolaserpitin[d]	0.77± 0.04	
Demethylsuberosin[e]	0.45± 0.02	
Selinidin[d]	0.56 ± 0.02	
Bergapten[f]	0.06 ± 0.01	

Coumarin	Concentration (mg/g dry weight)[a]	Structure
Imperatorin[g]	1.23 ± 0.09	
Pteryxin[h]	0.08 ± 0.01	

[a] Adapted from Kim et al. (2014)
[b] Structure from Sigma-Aldrich (www.sigmaaldrich.com/US/en/product/sigma/p8399?context=product)
[c] Structure from Sigma-Aldrich (www.sigmaaldrich.com/US/en/product/sial/56448?context=product)
[d] Structure from Akihisa et al. (2003)
[e] Structure from Sigma-Aldrich (www.sigmaaldrich.com/US/en/product/targetmoleculecorp/ta9h93cfc25b?context=bbe)
[f] Structure from Sigma-Aldrich (www.sigmaaldrich.com/US/en/product/sial/69664?context=product)
[g] Structure from Sigma-Aldrich (www.sigmaaldrich.com/US/en/substance/imperatorin27028482440?context=product)
[h] Structure from Sigma-Aldrich (www.sigmaaldrich.com/US/en/product/supelco/phl83899?context=product)

JBSL-USA analyzed the typical coumarin content of three non-consecutive lots of ashitaba yellow sap and found the average total coumarin content to be 38.62 mg per g. Results are summarized in Table 8.

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Table 8. Typical Coumarin Content in JBSL-USA's Ashitaba Yellow Sap

Compound	Representative Lot			
	Lot# IACC0910	Lot# IACD077	Lot# IACE0322	Average
	Concentration (mg/g)			
Linear-Type Furanocoumarins				
Xanthotoxin (Psoralen)	0.711	0.795	0.774	0.760
Isopimpinellin	0.792	0.937	0.928	0.882
Bergapten	0.205	0.260	0.211	0.225
Imperatorin	<0.001	<0.001	<0.001	<0.001
Total	1.70	1.99	1.91	1.87
Angular-Type Dihydropyranocoumarins				
Laserpitin	13.599	13.059	15.560	14.070
Isolaserpitin	15.193	14.404	19.534	16.377
Selinidin	5.694	5.655	7.544	6.298
Total	34.49	33.12	42.64	36.75
Total Coumarins	**36.19**	**35.11**	**44.55**	**38.62**

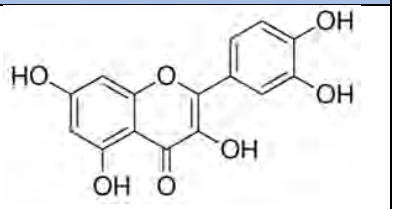
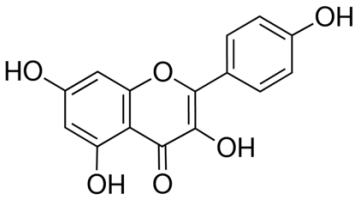
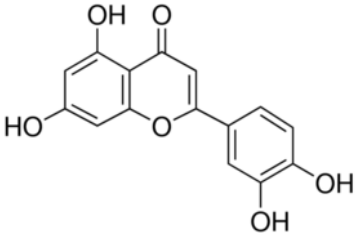
2.2.1.3 Flavones and Flavonols

Flavones and flavonones are present in the leaves, flowers, and fruits of many edible plants, including celery, red peppers, mint, onions, lettuce, tomatoes, and grapes.

Yang et al. (2008) analyzed the flavonoid content of 91 edible plant species, including ashitaba. The flavonols quercetin and kaempherol, as well as the flavone luteolin, were observed in *A. keiskei* shoots (Table 9); however, there have been no quantitative reports of the flavonone content of ashitaba stems or yellow sap in the published literature.

[Remainder of page is blank]

Table 9. Flavones and Flavonols Isolated from* Angelica keiskei *Shoots

		Concentration (mg/100 g fresh weight)[a]	Structure
Flavonols	**Quercetin**[b]	121.7	
	Kaempferol[c]	1.7	
Flavone	**Luteolin**[d]	95.8	

[a] Adapted from Yang et al. (2008)

[b] Structure from Sigma-Aldrich (www.sigmaaldrich.com/US/en/substance/quercetin30224117395?context=product)

[c] Structure from Sigma-Aldrich (www.sigmaaldrich.com/US/en/product/sigma/60010?context=product)

[d] Structure from Sigma-Aldrich (www.sigmaaldrich.com/US/en/product/sigma/l9283?context=product)

A summary of the flavanones identified in ashitaba stems or yellow sap reported in the published scientific literature to date is provided in Table 10.

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Table 10. Flavanones Extracted from Ashitaba Yellow Sap or Stem

Flavanone	Reference
4′-*O*-geranylnaringenin	Akihisa et al. (2003)
8-geranylnaringenin	Akihisa et al. (2006)
Isobavachin	Akihisa et al. (2006)
Mundulea flavanone A	Akihisa et al. (2003)
Mundulea flavanone B	Akihisa et al. (2006)
Prostratol F	Akihisa et al. (2003)

2.2.2 Ashitaba Chalcone Powder (8%) Composition

JBSL-USA's Ashitaba Chalcone Powder (8%) preparation is a yellow powder primarily composed of carbohydrates, fat, chalcones, and moisture. The typical chemical composition of Ashitaba Chalcone Powder (8%) is shown in Table 11.

Table 11. Typical Composition of Ashitaba Chalcone Powder (8%)

Component	Typical Content
Moisture	0.6%
Protein (as is, N X 6.25)	0.5%
Fat	9.4%
Ash	0.7%
Carbohydrate	88.8%
Energy	442 kcal/100 g
Total Chalcone[a]	8%
Xanthoangelol	5%
4-Hydroxyderricin	3%
Other Chalcones[b]	3.6%

[a] Reported as the sum of xanthoangelol and 4-hydroxyderricin

[b] Reported as the sum of isobavachalcone, and xanthoangelols B, C, D, E, F, and G

While *A. keiskei* leaves are known to contain carotenoids, analyses conducted by JBSL-USA on products derived from yellow sap demonstrate that no carotenoids—including α-carotene, β-carotene, lutein, zeaxanthin, and lycopene—are present. Vitamin K_1 has also been identified in *A. keiskei* leaves; however, no Vitamin K_1 was detected in two representative lots of JBSL-USA's Ashitaba Chalcone Powder (8%).

2.2.3 Summary

Ashitaba yellow sap is a dietary source of vitamins, minerals, amino acids, and lipids, as well as a variety of flavonoids, including chalcones (4-HD and XA), coumarins (xanthotoxin, imperatorin, and laserpitin), flavonols, and flavones.

2.3 Manufacturing Process

JBSL-USA cultivates our ashitaba plants on Indonesian farms owned and managed by Japan Bio Science Laboratory using organic farming techniques, where the soil is weeded, mulched with organic fertilizer, and the plants are protected against insects without the use of pesticides, resulting in an ashitaba sap raw material that meets the Japanese Agricultural Standard for organic certification. JBSL-USA manufactures Ashitaba Chalcone Powder (8%) under CGMP. Organic certification documents for the ashitaba sap raw material and ISO 9001:2015 certification documents are provided in Appendix 1.

The ISOELEAT P branched cyclodextrin, composed of >80% total cyclodextrins and >50% maltosyl-cyclodextrin with minor components including maltose and dextrin, is used to manufacture Ashitaba Chalcone Powder (8%). ISOELEAT P is derived from non-Genetically Modified (non-GMO) corn and potato starch. Supporting documentation is provided in Appendix 2.

Manufacturing Ashitaba Chalcone Powder (8%) begins with cutting the *A. keiskei* stems and harvesting the yellow sap exudate. The collected sap is pasteurized, mixed with ISOELEAT P at a ratio of 30% ashitaba sap to 70% branched cyclodextrin, and then sterilized at 121°C for 15 minutes. The mixture is then freeze dried and shattered before being passed through a 100 mesh to obtain the finished Ashitaba Chalcone Powder (8%) product.

A flow chart of the manufacturing process is provided in Figure 8 and an image of the Ashitaba Chalcone Powder (8%) finished product is shown in Figure 9.

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Figure 8. Ashitaba Chalcone Powder (8%) Manufacturing Flow Chart

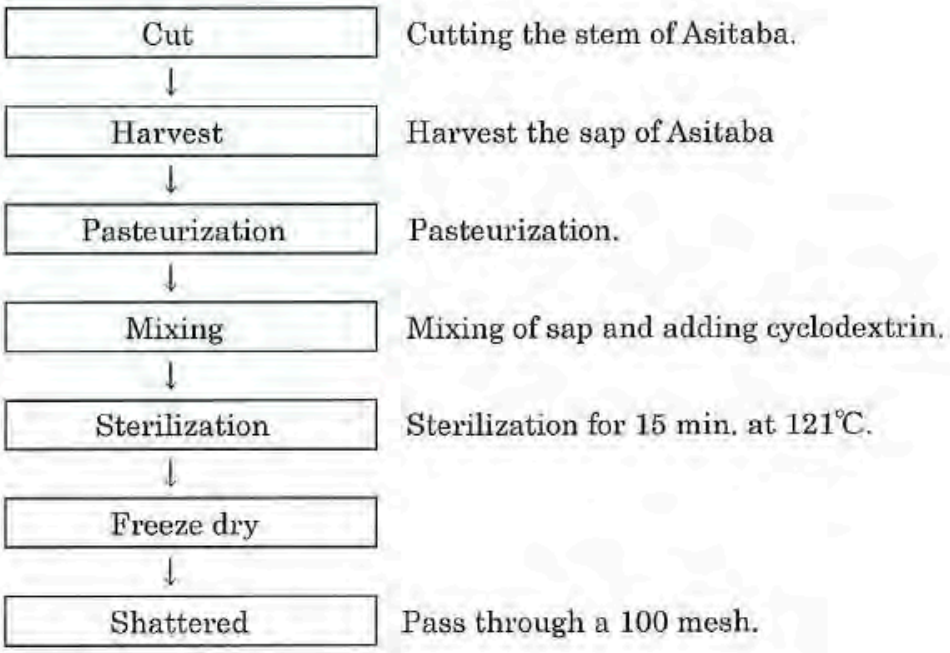

Figure 9. Ashitaba Chalcone Powder (8%)

2.4 Product Specifications

2.4.1 Accepted Specifications for Ashitaba Chalcones

There are no known specifications established for ashitaba or any ashitaba-derived preparations by international regulatory bodies, including the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the World Health Organization (WHO), the European Food Safety Authority (EFSA), or FDA.

2.4.2 Methods of Analysis

JBSL-USA uses microscopy and high performance thin-layer chromatography (HP-TLC) for botanical identity testing. Representative microscopy and HP-TLC analysis reports for the ashitaba stem raw material and a representative HP-TLC analysis report for Ashitaba Chalcone Powder are provided in

Appendix 3. Chalcone quantitation is conducted according to JBSL-USA's "Assay Method of Chalcone" using 4-HD and XA purified standards (Appendix 4).

2.4.3 Specifications for JBSL-USA's Ashitaba Chalcone Powder (8%)

The product specifications for JBSL-USA's Ashitaba Chalcone Powder (8%), along with analytical results from 5 non-consecutive representative lots, are shown in Table 12. Certificates of Analysis (COAs) for 5 non-consecutive batches of Ashitaba Chalcone Powder (8%) are provided in Appendix 5.

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Table 12. Representative Batch Analysis for JBSL-USA's Ashitaba Chalcone Powder (8%)

Physical and Chemical Parameters	JBSL-USA's Specification for Ashitaba Chalcone Powder (8%)	Manufacturing Batches					Method of Analysis
		Lot No. IACA6120	Lot No. IACA6628	Lot No. IACA7105	Lot No. IACA8521	Lot No. IACA9910	
Identity	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	HPLC
Total Chalcones[a] (%)	NLT 8.0	8.2	8.0	8.7	8.5	8.2	HPLC
Loss on drying (%)	NMT 8.0	2.1	1.9	1.4	2.5	2.9	1 g, 105°C, 4 hours
Arsenic (ppm)	NMT 1	NMT 0.1	NMT 0.1	NMT 0.1	NMT 0.1	NMT 0.1	ICP/MS
Cadmium (ppm)	NMT 1	0.003	0.002	0.002	NMT 0.004	NMT 0.01	ICP/MS
Lead (ppm)	NMT 1	NMT 0.01	0.016	0.016	NMT 0.01	NMT 0.05	ICP/MS
Mercury (ppm)	NMT 1	NMT 0.01	NMT 0.01	NMT 0.01	NMT 0.01	NMT 0.005	ICP/MS
Total Viable Aerobic Count (cfu per g)	NMT 1,000	NMT 300	NMT 300	NMT 300	NMT 300	NMT 300	US FDA BAM (Ch. 3)
***Salmonella* (per 25 g)**	Negative	Negative	Negative	Negative	Negative	Negative	AOAC 967.26
***E. coli* (per g)**	Negative	Negative	Negative	Negative	Negative	Negative	US FDA BAM (Ch. 4a)
Coliforms (cfu per g)	NMT 30	NMT 10	NMT 10	NMT 10	NMT 10	NMT 10	ISO 4382:1991
Yeast & Mold (cfu per g)	NMT 100	NMT 10	NMT 10	NMT 10	NMT 10	NMT 10	US FDA BAM (Ch. 18)

[a] Reported as the sum of xanthoangelol and 4-hydroxyderricin

AOAC –Association of Official Analytical Chemists; BAM – Bacteriological Analytical Manual; CFU – colony forming unit; g – gram; HPLC – high performance liquid chromatography; ICP/MS – inductively coupled plasms/mass spectrometry; ISO – International Organization for Standardization; NLT – not less than; NMT – not more than; ppm – parts per million

2.4.4 Pesticide Residue Analysis

Representative pesticides analysis reports for two lots of the ashitaba yellow sap raw material are provided in Appendix 6.

2.4.5 Conclusion

The collection of data presented in Part 2, as well as the supporting documentation in Appendices 1-6, demonstrates that JBSL-USA's Ashitaba Chalcone Powder (8%) preparation is well-characterized and meets appropriate specifications for food-grade materials.

2.5 Physical or Technical Effect

JBSL-USA's Ashitaba Chalcone Powder (8%) preparation is intended for use as an ingredient in conventional foods for consumers wishing to increase their dietary intake of the ingredient.

2.6 Stability

JBSL-USA conducted stability studies on the purified chalcones, XA and 4-HD, as well as on Ashitaba Chalcone Powder (8%).

2.6.1 Stability of Xanthoangelol and 4-Hydroxyderricin

Purified preparations of XA and 4-HD were observed to be stable over the course of 30 months when stored at both 5°C and at room temperature, as demonstrated in Figure 10 and Figure 11, respectively. Therefore, the approximate 2:1 ratio of XA and 4-HD is expected to remain constant in the Ashitaba Chalcone Powder (8%) over the course of 30 months when stored at room temperature.

Figure 10. Xanthoangelol and 4-Hydroxyderricin Stability at 5 ℃

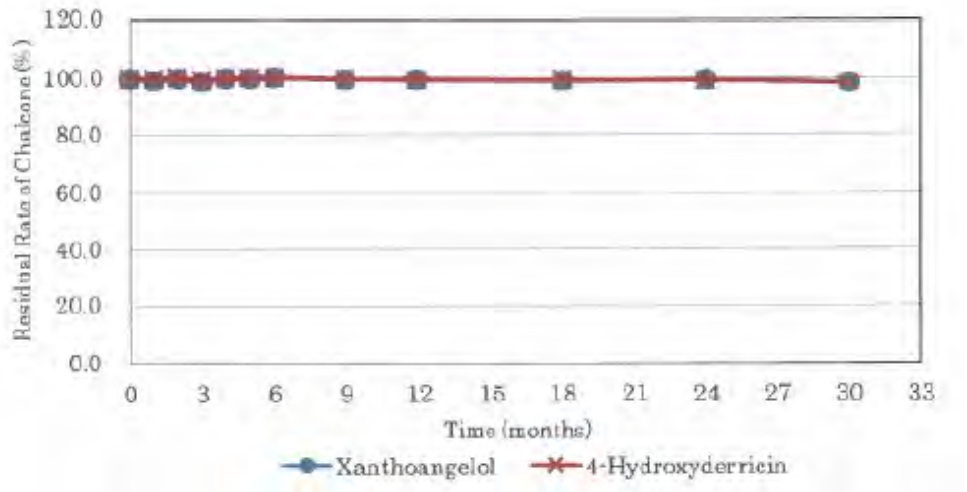
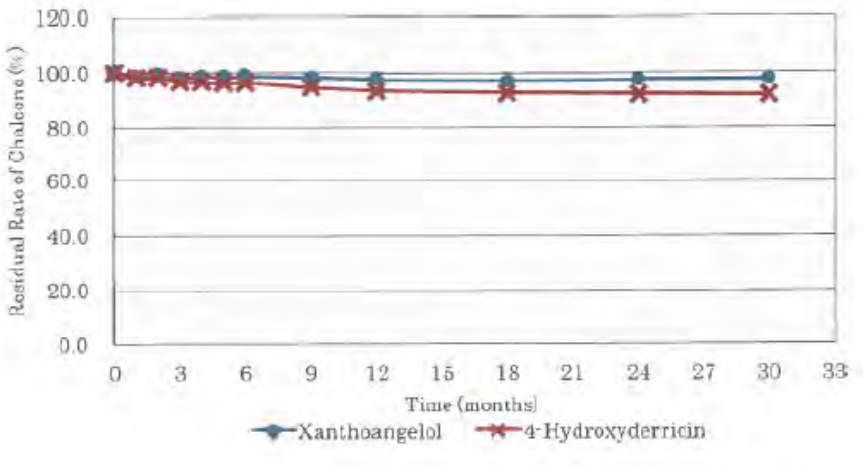


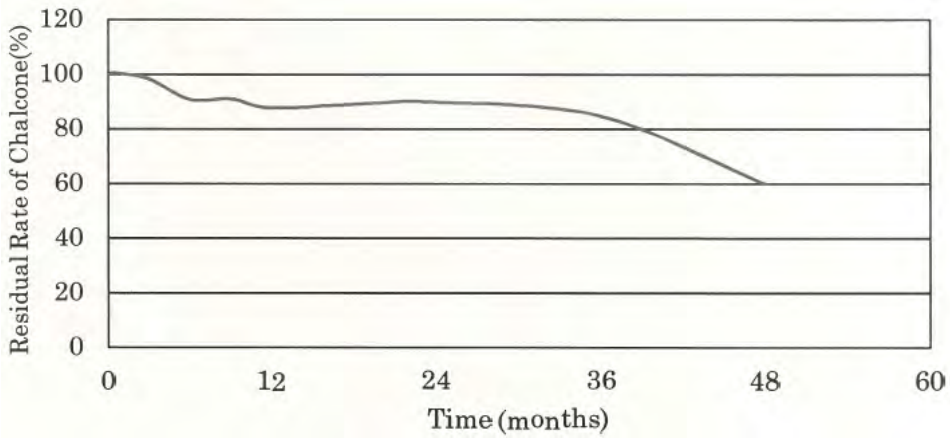
Figure 11. Xanthoangelol and 4-Hydroxyderricin Stability at Room Temperature



2.6.2 Stability of Ashitaba Chalcone Powder (8%)

A sample of Ashitaba Chalcone Powder (8%) was placed in an aluminum bag and stored at room temperature. The sample was tested at various time points between 0 and 48 months. The amount of total chalcone, as the sum of XA and 4-HD, was set as 100% in month 0 and the residue ratio of the total chalcones was calculated. JBSL-USA determined that Ashitaba Chalcone Powder (8%) is stable when stored at room temperature for 36 months, as determined by the measurement of total chalcones (Figure 12).

Figure 12. Ashitaba Chalcone Powder Stability at Room Temperature



2.6.3 Conclusion

JBSL-USA concludes that Ashitaba Chalcone Powder (8%) is stable for at least 36 months when stored at room temperature as demonstrated in Figure 12.

Part 3. Dietary Exposure

3.1 Intended Food Uses

JBSL-USA intends to use Ashitaba Chalcone Powder (8%) as an ingredient in conventional foods for the general population in the specified categories and at a maximum use level of 125 mg per day.

3.2 Estimated Daily Intake

3.2.1 Ashitaba Chalcone Powder (8%)

3.2.1.1 Ashitaba Chalcone Powder (8%) in the Background Diet

There are no known intake assessments of ashitaba or ashitaba-derived ingredients for US consumers. Products containing ashitaba—including tea and green juice—are currently available for purchase on Amazon, as are ashitaba seeds for consumers to grow their own plants.[2]

3.2.1.2 Ashitaba Chalcone Powder (8%) from Intended Food Uses

JBSL-USA intends to use Ashitaba Chalcone Powder (8%) in a limited number of food categories at the use levels provided in Table 13. The estimated mean and high total consumption was determined following FDA's methodology (FDA, 2006) and using United Stated Department of Agriculture (USDA) National Health and Nutrition Examination Survey (NHANES) 2015-2016 data. FDA methodology is recognized as a procedure that overestimates consumption. For the intake assessment, consumption data from individual dietary records were imported into Microsoft Excel and used to generate the estimated daily intake (EDI) for Ashitaba Chalcone Powder (8%) for consumers who reported consuming food products in which Ashitaba Chalcone Powder (8%) is proposed for use. Food codes from NHANES 2015-2016 were selected for each proposed food category according, as listed in Appendix 7.

[2] Kenkō Raw Ashitaba Tea, https://www.amazon.com/Kenk%C5%8D-Raw-Ashitaba-Tea/dp/B01B8OS88W/ref=sr_1_4?keywords=angelica+keiskei&qid=1576779601&sr=8-4 (Accessed April 30, 2021); Karuna Whole Plant Juice – Viva (Ashitaba, Beet & Pomegranate), https://www.amazon.com/Karuna-Whole-Plant-Juice-Pomegranate/dp/B07TGTBS6T/ref=sr_1_33?keywords=angelica+keiskei&qid=1576779298&sr=8-33 (Accessed April 30, 2021); Nana's Organic Ashitaba, Matcha style tea, https://www.amazon.com/dp/B07WFVS3S3/ref=dp_cerb_1 (Accessed April 30, 2021); Ashitaba Seeds, https://www.amazon.com/Elwyn-20-Ashitaba-Plant-Seeds/dp/B08WPP4WVM/ref=sr_1_1?dchild=1&keywords=ashitaba+seeds&qid=1619805519&s=lawn-garden&sr=1-1 (Accessed April 30, 2021)

Table 13. Daily Dietary Intake Estimations for Ashitaba Chalcone Powder

Food Category	Maximum Use Level (per serving)	RACC[a,b] Serving size (g)	USDA Mean Grams of Food Consumed (Consumer-only, per day)[e]	Mean mg Ashitaba Chalcone Powder Consumed (Consumer-only, per day)	Mean X 2 mg Ashitaba Chalcone Powder Consumed (Consumer-only, per day)
Soft Drinks	0.0075%	360	366	0.076	0.15
Jelly, Jams, Preserves, & Marmalade	0.834%	20[c]	29	12.1	24.2
Candy containing chocolate	0.006%	30	31	0.062	0.12
Butter	0.468%	14[d]	11	3.68	7.35
Total	--	--	--	15.9	31.8

[a] Reference amounts customarily consumed (RACC) as indicated by FDA, Available at: https://www.regulations.gov/document/FDA-2004-N-0258-0136 (Accessed on April 30, 2021)

[b] For liquids, assume 1 mL = 1 g

[c] For jelly, jams, preserves, & marmalade, assume 1 tbsp = 20 g (based on Welch's Concord Grape Jelly serving size)

[d] For butter, assume 1 tbsp = 14 g (based on Land O Lakes Salted Butter serving size)

[e] Average reported intake of individuals who reported consuming food products in which the use of Ashitaba Chalcone Powder is proposed on Day 1 and/or Day 2 of the survey.

The EDI for Ashitaba Chalcone Powder (8%) calculated by this method is approximately 32 mg per person per day. Considering that JBSL-USA's Ashitaba Chalcone Powder contains 8% chalcones, the maximum exposure to chalcones from JBSL-USA's proposed uses and use levels outlined in Table 13 would be approximately 2.56 mg chalcones per person per day.

In addition to the proposed uses specified in Table 13, Ashitaba Chalcone Powder (8%) may also be used as an ingredient in a variety of foods at a maximum use level of 125 mg per day. In the event a consumer followed a diet that preferentially included foods with the maximum use levels of Ashitaba Chalcone Powder (8%), that consumer would have a combined daily intake of 157 mg per day, which would provide the equivalent of 12.6 mg chalcones per person per day.

3.2.2 Acceptable Daily Intake

Using the NOAEL from Maronpot (2015) of 300 mg ashitaba chalcone powder per kg bw per day and a safety factor of 100, the Acceptable Daily Intake (ADI) for ashitaba chalcone powder in humans can be calculated as 3 mg ashitaba chalcone powder per kg bw per day or 210 mg ashitaba chalcone powder per person per day, using 70 kg as the reference body weight for humans. The ashitaba chalcone powder in the study was reported to contain 8.45% chalcones; therefore, the ADI can be extrapolated to be equivalent to 17.7 mg chalcones per person per day.

The highly conservative 'mean X 2' Estimated Daily Intake (EDI) of 32 mg per day combined with the maximum use level of 110 mg Ashitaba Chalcone Powder (8%) for the highest use consumers, for a total maximum consumption of 157 mg per day, is expected to be significantly lower than the calculated ADI of 210 mg ashitaba chalcone powder per person per day, based on Maronpot (2015).

3.2.3 Chalcones from Ashitaba Chalcone Powder

3.2.3.1 Current Dietary Exposure to Chalcones from the Background Diet

While it has been suggested that chalcones are an important class of flavones in the human diet, there are limited data available regarding chalcone content of common foods due to analytical difficulties. Studies have indicated that chalcone glycosides tend to convert to flavanone glycosides during the extraction process, and many analytical techniques convert the chalcone to the flavanone via acid hydrolysis prior to HPLC analysis (Tomás-Barberán and Clifford, 2000). No publications regarding the chalcone content of leafy green vegetables, including spinach and kale, were identified.

The greatest dietary contributor of chalcones in the US diet is likely tomatoes, where up to 0.7 mg chalcones could be consumed for every 100-g portion of the flesh and skin (Tomás-Barberán and Clifford, 2000). A summary of chalcones identified in common foods is provided in Table 14.

The Flavor and Extract Manufacturers Association (FEMA) and JECFA have found a number of chalcones to be GRAS for use as flavoring agents: naringin dihydrochalcone (FEMA No. 4495, JECFA No. 2208); hesperetin dihydrochalcone (FEMA No. 4872); neohesperidin dihydrochalcone (FEMA No. 3811), naringin dihydrochalcone aglycone (FEMA No. 4390, JECFA No. 2022), and prunin dihydrochalcone (FEMA No. 4674, JECFA No. 2171) (Marnett et al., 2013; Cohen et al., 2018; Smith et al., 2011; JECFA, 2014; 2010; 2012).

Table 14. Chalcones in Common Foods

Chalcone	Dietary Source	Reported Content	Reference
Naringenin chalcone	Tomato skin	64 mg/kg fresh weight	Tomás-Barberán and Clifford (2000)
		552 mg/kg fresh weight (ripe fruit)	Iijima et al. (2008)
Eriodictyol chalcone	Tomato skin	17.3 mg/kg fresh weight (ripe fruit)	Iijima et al. (2008)
Echinatin	Licorice root	N/A	Tomás-Barberán and Clifford (2000)
Licochalcones A & B	Licorice root	N/A	Tomás-Barberán and Clifford (2000)
Xanthohumol	Hops & Beer	N/A	Tomás-Barberán and Clifford (2000)
Desmethylxanthohumol	Hops & Beer	N/A	Tomás-Barberán and Clifford (2000)
Phloridzin	Apples	5-10 mg/kg for most cultivars, but can range from 0.1 mg/kg to as high as 190 mg/kg	Tomás-Barberán and Clifford (2000)
Phloretin 2′(2″-xylosyl-glucoside)	Apple flesh	10-30 mg/kg	Tomás-Barberán and Clifford (2000)
3-hydroxyphloridzin	Apple pomace	270 mg/kg	Tomás-Barberán and Clifford (2000)
Phloretin 2-xyloglucoside	Spanish cider apple juice	26-36 mg/L	Tomás-Barberán and Clifford (2000)
Total flavanone/chalcone	Beer sold in the US	0-4 mg/L	Tomás-Barberán and Clifford (2000)

3.2.3.2 Chalcone Consumption for Ashitaba Chalcone Powder (8%) from Intended Uses

The 8% chalcone content specification established by JBSL-USA for Ashitaba Chalcone Powder was used to approximate the EDI of chalcones from the consumption of Ashitaba Chalcone Powder (8%). The combined intake of 157 mg per day Ashitaba Chalcone Powder (8%) would provide the equivalent of 12.6 mg chalcones per day, which is also below the extrapolated ADI for chalcones of 17.7 mg per day based upon Maronpot (2015).

3.2.3.3 Cumulative Intake of Chalcones from Background Diet and Ashitaba Chalcone Powder (8%)-Containing Foods

There have been no published estimates of overall chalcone consumption in Western diets, therefore it is impossible to estimate the cumulative intake of chalcones from the background diet and from the

proposed uses of Ashitaba Chalcone Powder (8%). The chalcones identified in common foods, such as tomatoes, apples, and hops are not reported to be present in *A. keiskei*, nor have the two main chalcones found in ashitaba (4-HD and XA) been reported in other foods. Based on the available published data, there is no reason to presume that dietary chalcones are functionally equivalent or bioactive.

3.2.4 Coumarins from Ashitaba Chalcone Powder (8%)

3.2.4.1 Current Dietary Exposure to Coumarins from the Background Diet

Coumarins are found in many conventional foods, including green plants, fungi, fruits, green tea, and spices. However, there are limited data available regarding the consumption levels of coumarins from conventional foods. Many published studies focus on coumarin, which is present in Ceylon and Cassia cinnamon at levels of up to 297 and 7,670 mg per kg powder, respectively (Borges Bubols et al., 2013). EFSA reviewed available toxicity data and determined the tolerable daily intake of coumarin from dietary sources is 0.1 mg per kg body weight (bw) (EFSA, 2008).

3.2.4.2 Coumarin Consumption from Ashitaba Chalcone Powder (8%) from Intended Uses

Based on quantitative data on ashitaba yellow sap obtained by JBSL-USA (see Table 8), there are approximately 38.6 mg total coumarins per g ashitaba yellow sap. The estimated total coumarin content in ashitaba yellow sap can be used to approximate the coumarin content in Ashitaba Chalcone Powder (8%), which is composed of 30% ashitaba yellow sap. Therefore, it is estimated that there are 11.6 mg coumarins per g Ashitaba Chalcone Powder (8%). A consumer with a cumulative Ashitaba Chalcone Powder (8%) intake of 157 mg per day would consume approximately 1.8 mg coumarins.

3.2.4.3 Cumulative Intake of Coumarins from Background Diet and Ashitaba Chalcone Powder (8%)-Containing Foods

There have been no published estimates of total coumarins consumption in Western diets, therefore it is impossible to estimate the cumulative intake of coumarins from the background diet and from the proposed uses of Ashitaba Chalcone Powder.

Assuming an average weight of 70 kg for adults, the estimated intake of coumarins from 157 mg Ashitaba Chalcone Powder would be 0.026 mg per kg bw per day. This is lower than the tolerable daily intake of coumarin from dietary sources of 0.1 mg per kg bw (EFSA, 2008). Based on the available published data, there is no reason to presume that dietary coumarins are functionally equivalent or bioactive. Furthermore, coumarin has not been reported in *A. keiskei* yellow sap.

3.2.5 Flavones and Flavonols from Ashitaba Chalcone Powder (8%)

3.2.5.1 Current Dietary Exposure to Flavones and Flavonols from the Background Diet

Flavones and flavonols are present in the leaves, flowers, and fruits of many edible plants, including celery, onions, lettuce, tomatoes, grapes, red peppers, and mint.

Using the USDA Flavonoid Database and 24-hour dietary recall data from NHANES 1999-2002, Chun et al. (2007) estimated the mean intake for flavonols (quercetin, kaempferol, myricetin, and isorhamnetin) and flavones (luteolin and apigenin) to be 12.9 and 1.6 mg per day for men and women aged 19 years and older, respectively. Mean flavonol intake from vegetables and vegetable products ranged from 5.5 mg per day for non-consumers to 19.2 mg per day for consumers in the upper third tertile, while mean flavone intake ranged from 0.0 mg per day for non-consumers to 2.0 mg per day for consumers in the upper third tertile based upon NHANES 1999-2000 data.

More recently, Birt and Jeffery (2013) reported flavonoid intake in the human diet ranging from 20-200 mg per day, with tea drinkers ingesting as much as 1,000 mg flavonoids per day.

Quercetin is GRAS at use levels of up to 500 mg per serving in beverages and beverage bases, grain products and pastas, processed fruits and fruit juices, and soft candies (GRN 341). Background consumption was estimated to be 5.9 mg per person per day and 14.7 mg per person per day at the mean and 90th percentile for the total US population, respectively, with a maximum intake of 258 mg per person per day (Quercegen, 2010).

3.2.5.2 Flavones and Flavonols Consumption from Ashitaba Chalcone Powder (8%) from Intended Uses

The flavone luteolin and the flavonols quercetin and kaempferol were identified in the aerial portions of *A. keiskei*; however, no quantitative information is available in the published literature for content in ashitaba yellow sap. The estimated daily intakes of quercetin, kaempferol, and luteolin from the consumption of Ashitaba Chalcone Powder (8%), which is composed of 30% ashitaba yellow sap, was determined based upon reported concentrations in *A. keiskei* shoots (see Table 9), as shown in Table 15.

Table 15. Estimated Daily Intakes of Flavones and Flavonols from Ashitaba Chalcone Powder

Population Group	Estimated Ashitaba Chalcone Powder Intake (mg/day)	Estimated Quercetin Intake from Ashitaba Chalcone Powder (mg/day)	Estimated Kaempferol Intake from Ashitaba Chalcone Powder (mg/day)	Estimated Luteolin Intake from Ashitaba Chalcone Powder (mg/day)
Total Population	157	0.06	0.0008	0.05

3.2.5.3 Cumulative Intake of Flavones and Flavonols from Background Diet and Ashitaba Chalcone Powder (8%)-Containing Foods

Flavones and flavonols are found in many conventional foods, including green plants, fungi, fruits, green tea, and spices. However, there are limited and varying data available regarding the

consumption levels of flavones and flavonols from conventional foods in the US. Given the ubiquitous nature of flavonoids, and similar levels of quercetin, kaempferol, and luteolin reported for ashitaba shoots by Yang et al. (2008) and those reported by USDA in select foods, as compared in Table 16, the addition of Ashitaba Chalcone Powder (8%) to the diet under the proposed uses and use levels is not expected to pose a safety concern.

Table 16. Reported Flavone and Flavanone Content of Conventional Foods

Food	Quercetin (mg/100 g)	Kaempferol (mg/100 g)	Luteolin (mg/100 g)
Ashitaba shoot[a]	121.7	1.7	95.8
Lovage, leaves, raw[b]	170.00	7.00	0.00
Onions, raw (*Allium cepa*)[b]	1.50-90.75	0-1.41	0-0.19
Onions, red, raw[b]	5.90-191.70	0-4.50	0-1.10
Radicchio, raw (*Cichorium intybus*)[b]	9.06-52.73	NR	16.60-77.27
Radish leaves[b]	70.37	7.72	NR
Rocket, wild, raw (*Diplotaxis tenuifolia*) [b]	66.19	1.78	NR

[a] Adapted from Yang et al. (2008)

[b] Adapted from USDA (2018), edible portion of plants

NR – Not reported

3.2.6 Summary

The EDI for JBSL-USA's Ashitaba Chalcone Powder (8%) was calculated based on proposed uses in soft drinks, fruit preserves, candy containing chocolate, and butter, at the use levels detailed in Table 13, as well as proposed use as an ingredient in conventional foods at a maximum use level of 110 mg per day. The mean total EDI for all individuals was determined to be 15.9 mg Ashitaba Chalcone Powder (8%), and the highly conservative 'mean X 2' was determined to be 31.8 mg Ashitaba Chalcone Powder (8%). The maximum cumulative intake from all proposed uses for the highest consumers is 157 mg Ashitaba Chalcone Powder (8%) per person per day. No appreciable daily intake of ashitaba or ashitaba-derived ingredients is anticipated from the background diet; therefore, no cumulative intake of ashitaba from the background diet and proposed uses can be determined.

The ADI for ashitaba chalcone powder of 3 mg per kg bw per day was calculated using a NOAEL of 300 mg per kg bw per day and a safety factor of 100, which is equivalent to 210 mg per person per day (using 70 kg as the reference body weight for humans). The mean and 'mean X 2' EDI, as well as the

maximum cumulative intake from all proposed uses, fall well-below the calculated ADI for Ashitaba Chalcone Powder (8%) at 15.9 g, 31.8 g, and 157 mg, respectively.

Furthermore, intake assessments for chalcones, coumarins, flavones, and flavonones identified in *A. keiskei* do not raise any cumulative intake safety concerns for the proposed uses and use levels of JBSL-USA's Ashitaba Chalcone Powder (8%) as described herein.

3.3 Probable Consumption of Other Substances Formed In or On Food

No other substances are expected to be formed on or in food containing Ashitaba Chalcone Powder (8%) under the intended conditions of use.

3.4 Dietary Exposure to Contaminants or By-Products

There are no known concerns regarding dietary exposure to potential contaminants or by-products of Ashitaba Chalcone Powder (8%).

Part 4. Self-Limiting Levels of Use

There are no known self-limiting levels of use for Ashitaba Chalcone Powder (8%).

Part 5. Experience Based on Common Use in Food Before 1958

There are no known common uses of *A. keiskei* or Ashitaba Chalcone Powder in foods in the United States prior to 1958.

Part 6. Narrative

6.1 Introduction

A search of international regulatory actions on "ashitaba" and "*Angelica keiskei*" was conducted to identify any relevant food use assessments. An online literature search was conducted using Google Scholar and PubMed, current to May 17, 2021, using the terms "*Angelica keiskei*" and "ashitaba" to identify references with relevant safety data on Ashitaba Chalcone Powder (8%). In addition, publications regarding the two major active compounds in ashitaba, 4-hydroxyderricin (4-HD) and xanthoangelol (XA), were also reviewed for relevant safety data. No confidential or non-public safety-related data were reviewed.

6.2 History of Use in Foods

6.2.1 History of Traditional Medicinal and Human Food Use

Ashitaba is native to Japan and has been consumed there as a vegetable for many years (Enoki et al., 2010; Li et al., 2009; Maronpot, 2015; Ohkura et al., 2018; Ohkura et al., 2011; Zhang et al., 2019). It has been reported that it was a staple of the Samurai diets for millennia (Knapton, 2019). In addition to Japan, ashitaba is cultivated and consumed in other Asian countries including Korea, China, and Taiwan (Ohkura et al., 2018). Fuji Keizai (2011) reported that ashitaba leaf powder consumption averaged 149 metric tons between 2006 and 2011.

Today, ashitaba fresh leaves and dried powder are consumed as vegetables and as ingredients in Japanese foods, including ashitaba tea, cakes, powder, candy, and a confectionary called yatshuhashi (Akihisa et al., 2006; Akihisa et al., 2003; Inamori et al., 1991; Kim et al., 2014; Maronpot, 2015; Takaoka et al., 2008). It is available in Tokyo restaurants that serve local agricultural products (Tokyo Restaurants Guide, 2019). On the Izu island of Hachijojima, ashitaba is used as an ingredient in soba, ice cream, green beer, and tempura, and is also prepared as a vegetable by boiling and serving with soy sauce (Japanistry.com, 2019; Foster, 2005). On Hachijo Island, ashitaba is often cooked as tempura or boiled as a vegetable, and 50 to 100 grams of leaves are eaten. Nagata et al. (2007) estimated that there are 200 to 300 mg total chalcones per 100 grams of ashitaba leaf. Therefore, each ashitaba serving would contain approximately 100 to 300 mg chalcones.

Ashitaba was included in a dietary intake study conducted by the Japanese Ministry of Health, Labour and Welfare (JMHLW). Though the raw survey data is not provided, the results are available on JMHLW's website.[3] The study surveyed the individual food intakes of 40,394 individuals, and the reported average consumption of ashitaba preparations are summarized in Table 17.

Ashitaba is also commonly found as an ingredient in aojiru, a health food made from green vegetables such as ashitaba, kale, mulberry leaf, and barley grass (Shibano et al., 2009; Sakamaki et al., 2006). In a 2006 NPI Center article regarding the Japanese nutraceutical market, aojiru products were reported to be "often made with kale and most recently ashitaba" and were the third most popular products on Kenco.com (a popular Japanese e-drugstore) in 2005 (NPI Center, 2006).

[3] Available at: https://www.mhlw.go.jp/english/topics/foodsafety/foodadditives/dl/syokuhinsessyuhindo-english.pdf (Accessed on June 25, 2021)

Table 17. Average Intakes of Ashitaba by Japanese Consumers[a]

Population Group	Age Group	Average weight (kg)	Number of individuals surveyed	Average intake of Angelica, "Ashitaba" (g)	Average intake of Angelica, "Ashitaba", boiled (g)
Children	1-6 years (avg. 3.8 years)	16.5	1,619	0.033	0.000
	7-14 years (avg. 10.6 years)	37.9	3,419	0.000	0.000
	15-19 years (avg. 16.8 years)	55.9	2,542	0.000	0.000
Adults	Aged >20 years (avg. 53.2 years)	58.8	32,814	0.042	0.042
Pregnant women	Avg. 27.4 years	58.5	77	0.000	0.000
Nursing women	Avg. 33.7 years	54.5	83	0.000	0.000
Total Population	Avg. 45.4 years	55.1	40,394	0.035	0.034

[a] Adapted from Japanese Ministry of Health (Date Unknown).

6.2.2 Summary of the Regulatory History of Ashitaba

6.2.2.1 United States Regulatory History

A search of FDA's GRAS Notice Inventory[4] website using the terms "ashitaba" and "Angelica keiskei" indicated that no GRAS notifications for ashitaba preparations have been filed by FDA as of May 17, 2021.

FDA's Poisonous Plant Database, lasted updated in May 2008, includes an entry for "asitaba" under Germplasm Resources Information Network (GRIN)#405622. FDA's disclaimer for the database indicates that the contents are for "scientific exchange" and provides access to studies and reports of toxic "properties and effects on plants and plant parts" found in print literature through 2007. Furthermore, FDA states the database is "neither error-free nor comprehensive." It should be noted that the article reference for "asitaba" was for a study in which 19 plants were screened for large quantities of condensed tannins, which have been shown to inhibit trypsin activity. *A. keiskei* was not found to contain condensed tannins (Horigome et al., 1988).

6.2.2.2 Canadian Regulatory History

A search of Health Canada's website[5] using the terms "ashitaba" and "Angelica keiskei" indicated no regulatory actions have occurred as of May 17, 2021.

[4] Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices (Accessed on May 17, 2021).

6.2.2.3 European Regulatory History

The European Commission has summarized an application for ashitaba sap powder, submitted by JBSL-USA, per the requirements of the Novel Food regulation and Article 10(1) of Regulation (EU) 2105/2283. The ashitaba sap powder, a blend of sap derived from *A. keiskei* and cyclodextrin as an excipient (European Commission, 2019). A search of the European Commission's website[6] using the search terms "ashitaba" and "Angelica keiskei" did not identify any regulatory approvals.

A search of the European Food Safety Authority (EFSA) website[7] using the terms "ashitaba" and "Angelica keiskei" indicated no regulatory actions have occurred as of May 17, 2021.

A search of the United Kingdom's Food Standards Agency (FSA) website[8] using the terms "ashitaba" and "Angelica keiskei" indicated no regulatory actions have occurred as of May 17, 2021.

6.2.2.4 Asian Regulatory History

"Angelica" is listed as an approved source of spice extract and tannin extract and as an approved plant source of natural flavorings by the Japanese Ministry of Health and Welfare (Japanese Ministry of Health and Welfare, 1996; 2014); while unspecified, this may be a reference to *Angelica archangelica*, which is a different species.

The Japanese Ministry of Health and Welfare issued a report in May 2013 detailing the levels of radioactive contaminants in foods. Along with other common vegetables and fruits, ashitaba was listed in the "pre-marketed agricultural products" category. Ashitaba samples ("food origin" as Tochigi Prefecture, Nasu-machi) were reported to have radioactive contamination levels of 4.1 Becquerel (Bq) per kg for total cesium (Cesium-134 and Cesium-137) (Japanese Ministry of Health, 2013). These levels do not exceed the standard limits established by the Ministry of Health and Welfare (Japanese Consumer Affairs Agency, 2013; 2018). FDA's level of concern for Cesium-134 and Cesium-137 in infant food and other food is 370 Bq per kg and the derived intervention levels are 1,200 Bq per kg (FDA, 2004).

Ashitaba stem and leaf was officially approved as a new food raw material by Announcement No. 2 of 2019 by the National Health Commission of the People's Republic of China (NHC). Recommended daily intake is ≤ 50 g per day, where dry product consumption should be calculated based on fresh product levels (CIRS, 2020).

A search of Korea's Ministry of Food and Drug Safety website[9] using the terms "ashitaba" and "Angelica keiskei" indicated no relevant regulatory actions have occurred as of May 17, 2021.

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[5] Available at: https://www.canada.ca/en/health-canada.html (Accessed on May 17, 2021)
[6] Available at: https://ec.europa.eu/food/safety/novel_food_en (Accessed on May 17, 2021)
[7] Available at: http://www.efsa.europa.eu/ (Accessed on May 17, 2021)
[8] Available at: https://www.food.gov.uk/ (Accessed on May 17, 2021)

6.2.2.5 Australian/New Zealand Regulatory History

A search of the Food Standards Australia New Zealand (FSANZ) website[10] using the terms "ashitaba" and "Angelica keiskei" indicated no relevant regulatory actions have occurred as of May 17, 2021.

In an Initial Assessment Report and a Draft Assessment Report for Proposal P291 Review of Novel Food Standard, FSANZ noted the inclusion of *A. keiskei* in a Korean supplement drink that also contained *Artemisia princes, Ganoderma lucidum,* and *Cordyceps*. While the supplement drink was deemed to contain non-traditional and novel foods ingredients, no application for use was submitted (FSANZ, 2004; 2005). In the 2020 update to the "Record of views formed in response to inquiries," FSANZ listed the ashitaba-containing Korean supplement drink as a non-traditional and novel food, with a note stating: "Safety concerns [are] based on potential for adverse effects in humans." FSANZ did not specify the nature of the safety concerns or which ingredient or ingredients were of concern (FSANZ, 2020).

6.3 Absorption, Distribution, Metabolism, and Excretion (ADME)

6.3.1 ADME Studies on Ashitaba Chalcones

Chalcones are open chain flavonoids (1,3-diaryl-2-propen-1-ones) that are ubiquitous in the plant kingdom (Batovska and Todorova, 2010). The two most prevalent chalcones in JBSL-USA's Ashitaba Chalcone Powder (8%) are 4-HD and XA. Therefore, ADME studies on 4-HD and XA are relevant to the safety evaluation of JBSL-USA's Ashitaba Chalcone Powder (8%).

Nakamura et al. (2012) investigated the absorption and metabolism of 4-HD and XA from oral administration of ashitaba extract in mice. After acclimatization on commercial food and tap water for one week, seven-week-old mice (male, number unknown) were administered via gastric intubation a single dose of one of the following: 29 mg per kg bw 4-HD; 35 mg per kg bw XA; 50, 100, 200, or 500 mg per kg bw ashitaba extract (prepared from ashitaba sap powder and containing 145 µg per mg 4-HD and 173 µg per mg XA, on a dry weight basis); or 5% polysorbate 80 (vehicle control). The administered concentrations of purified 4-HD and XA were equivalent to those in the 200 mg per kg dose of ashitaba extract.

Plasma concentrations of 4-HD and XA at 2 hours post-administration of ashitaba extract increased at doses up to 200 mg per kg bw and decreased at the 500 mg per kg bw dose level. The plasma concentrations of mice administered the 200 mg per kg bw dose of ashitaba extract at 0.5, 1, 2, 4, 6, and 24 hours post-administration showed that both free and conjugated 4-HD and XA were present. Maximum plasma concentrations of free and conjugated 4-HD occurred at 2 hours post-

[9] Available at: https://www.mfds.go.kr/eng/search/searchEn.do (Accessed on May 17, 2021)
[10] Available at: https://www.foodstandards.gov.au/Pages/default.aspx (Accessed on May 17, 2021)

administration of ashitaba extract, while maximum plasma concentrations of free and conjugated XA were observed at 0.5 and 1 hour post-administration. The area under the curve for the 24-hour time period post-administration of 200 mg per kg bw ashitaba extract indicated that the total plasma concentration of 4-HD and its metabolites was approximately 4 times greater than the total plasma concentration of XA and its metabolites.

A decreased time to maximum plasma concentration was observed for the individual 4-HD and XA doses, at 1 hour and 0.5 hour, respectively. However, no difference in the total levels of absorbed 4-HD or XA were observed between mice administered the purified chalcones compared to the ashitaba extract.

Urinary concentrations of 4-HD and XA were measured at 2, 4, 8, and 24 hours post-administration of 200 mg per kg bw ashitaba extract. Fecal samples were collected at 24 hours post-administration. Urinalysis found that 4-HD, XA, and their metabolites were primarily excreted in their aglycone forms, as glucuronate and/or sulfate, between 2 and 8 hours post-administration. In the feces, the aglycone forms were prevalent, with only 0.4% 4-HD and 0.4% XA excreted in their intact form.

The concentrations of 4-HD and XA were measured in the liver, kidney, spleen, muscle, and white adipose tissue (perirenal, epididymal, and mesenteric fat) to assess tissue distribution. The aglycone forms of both 4-HD and XA were detected in all tissues examined except for mesenteric adipose tissue, with 4-HD present primarily in the adipose tissues and XA present primarily in the liver and mesenteric fat. The authors concluded that 4-HD and XA are rapidly absorbed and distributed to the tissues.

6.3.2 ADME Conclusion

JBSL-USA has reviewed these ADME studies, and concludes that they do not raise any concerns about the safety of Ashitaba Chalcone Powder (8%) when used as proposed herein. Furthermore, the anticipated daily intake levels of total chalcones, as summarized in Part 3, is significantly lower than the single dose of chalcones administered to mice (corresponding to 0.18 mg per kg total chalcones for a 70-kg adult vs. 64 mg per kg total chalcones for a mouse).

6.4 Published Pivotal Safety Studies

6.4.1 Acute Toxicity Studies

Maronpot (2015) studied the acute oral toxicity of an Ashitaba Chalcone Powder (8% chalcone, JBSL-USA) produced from *A. keiskei* sap. The study followed the Organisation for Economic Co-operation and Development (OECD) Guideline 423 and was conducted following Good Laboratory Practice (GLP) procedures. Six-week-old outbred Wistar rats (three male, three female) were given one dose of 2,000 mg Ashitaba Chalcone Powder per kg bw via gavage, which is equivalent to 160 mg chalcones

per kg bw. The rats were fasted overnight prior to dosing and four hours after dosing. Clinical signs, body weights, and macroscopic examination data were collected, and animals were observed for mortality and clinical signs for up to 14 days post-treatment. No mortality, clinical signs, or gross, treatment-related changes were observed at the 2,000 mg per kg dose level. Maronpot concluded that the median lethal dose (LD_{50}) of Ashitaba Chalcone Powder is higher than 2,000 mg per kg bw since no mortality was observed at that dose level.

6.4.2 Subacute Toxicity Studies

No subacute toxicity studies were identified in the published literature.

6.4.3 Subchronic Toxicity Studies

Maronpot (2015) investigated the effects of oral administration of an Ashitaba Chalcone Powder (8.45% chalcones, JBSL-USA) produced from *A. keiskei* sap in a 13-week repeated dose study in six-week-old Sprague Dawley rats. The study adhered to the Japanese Ministry of Health and Welfare Ordinance No. 21 and Notification No. 424 for Conduct of Non-Clinical Studies on the Safety of Drugs. Rats (12 males and 12 females per dose group) were administered olive oil (vehicle control) or Ashitaba Chalcone Powder at doses of 100, 300, or 1,000 mg per kg bw per day via gavage in a volume of 5 mL per kg bw. The rats were examined for clinical parameters, including appearance, nutritional condition, posture, behavior, excrement before, immediately following, and two hours after treatment or immediately before or after treatment. Necropsy, organ weight determination, and histopathological analyses were conducted immediately on animals found dead during the study and those animals euthanized at the end of the study. Body weight was measured on days 1, 4, and 7 of the first week of treatment, after which it was measured at 3-4 day intervals prior to dosing. Food consumption was measured three times in the first week of the study, and at subsequent 3-4 day intervals. Ophthalmological examinations were conducted before the first dosing and during the final week of the study. Urinalysis was conducted during the final week of the study, and hematological analysis was conducted one day after the last day of treatment administration.

No treatment-related deaths were reported. One male from the 300 mg per kg bw per day Ashitaba Chalcone Powder group died at week 10 due to intubation error and one male from the control group died at week 12 with disseminated erythroblastic leukemia. No toxicologically-significant findings were reported for body weight gain, food consumption, ophthalmological examination, or urinalysis. At the conclusion of the study, hematological analysis revealed reduced hemoglobin in males in the 1,000 mg per kg bw per day dose group; however, the values remained within normal physiological range and related erythrocyte counts and hematocrit values were also observed within normal physiological range. Decreased platelet counts and increased coagulation indices observed in both sexes were considered "marginal and not of toxicological concern" in the absence of clinical signs.

An increase in alkaline phosphatase (ALP) in males and females in the 1,000 mg per kg bw per day dose group was observed. As no significant change in alanine aminotransferase (ALT) concentration

and no significant increase in hepatocyte necrosis were observed, the increased level of ALP is not thought to indicate liver toxicity. An increase in aspartate aminotransferase (AST) was observed for females in the 1,000 mg per kg bw per day dose group; however, the large standard deviation indicates the result is a potential outlier. Furthermore, small changes in ASP concentration are frequently observed in non-clinical studies and are usually not considered adverse. Elevations in total cholesterol and phospholipids were observed for males and females in the 1,000 mg per kg bw per day dose group and triglyceride concentrations were also increased in males in the 1,000 mg per kg bw per day dose group. Chalcones have been shown to increase cholesterol transport and circulating triglycerides (Nagata et al., 2007; Ogawa et al., 2003); therefore, elevated serum levels of total cholesterol, triglyceride, and phospholipids were not unexpected.

Minimal elevations in blood urea nitrogen (BUN) concentration for males and females in the 1,000 mg per kg bw per day dose group were observed, but the values were within normal limits and there was no concurrent increase in serum creatinine or changes in urinary measurements; therefore, the BUN concentration was not considered toxicologically significant. In addition, while sporadic reductions in sodium and potassium were observed in high-dose males, the levels were within the expected electrolyte range for rats (Smith et al., 2013). It is possible that these observations reflect small homeostatic effects related to small differences in the fluid balance and food consumption that are not considered to be toxicologically significant (Hall, 2013). Elevations in the globulin fractions were observed for males and females in the 1,000 and 3,000 mg per kg bw per day dose groups, though the significance of these observations is questionable given the seemingly unremarkable total protein, albumin, and A/G ratios.

Histopathological analyses revealed changes in the adrenal glands, cecum, jejunum, kidney, liver, and stomach. The adrenal cortices of two male rats and three female rats in the 1,000 mg per kg bw per day dose group displayed minimal elevation in cytoplasmic vacuolation. Cellular infiltration of the cecal lamina propria was observed for 11 males (one in the control group; one in the 100 mg per kg bw per day group; four in the 300 mg per kg bw per day group; and five in the 1,000 mg per kg bw per day group) and for three females (one in the control group and two in the 1,000 mg per kg bw per day group). In the jejunum, lymphangiectasia (dilated lacteals) were observed for the majority of high dose males (n=11) and females (n=9). An increase relative weight of the kidney was observed for rats in the 1,000 mg per kg bw per day dose group. Apparent dose-related renal tubular alterations (alpha 2-urinary globulin nephropathy) was observed for 29 male rats (seven in the 100 mg per kg bw per day, 10 in the 300 mg per kg bw per day, and 12 in the 1,000 mg per kg bw per day dose groups, respectively) and no female rats. In the liver, cytoplasmic vacuolation of periportal hepatocytes was observed in 24 male rats (11 in the control group, six in the 100 mg per kg bw per day group, three in the 300 mg per kg bw per day group, and four in the 1,000 mg per kg bw per day group) and 15 female rats (four in the control group, one in the 100 mg per kg bw per day group, two in the 300 mg per kg bw per day group, and eight in the 1,000 mg per kg bw per day group). In addition, diffuse hepatocyte cytoplasmic vacuoles were observed in 27 male rats (five in the 100 mg per kg bw per day group, 11 in

the 300 mg per kg bw per day group, and 11 in the 1,000 mg per kg bw per day group) and 13 female rats (two in the 300 mg per kg bw per day group and 11 in the 1,000 mg per kg bw per day group). The stomach showed minimal or mild thickening of the limiting ridge for one female in the 300 mg per kg bw per day dose group, and four males and six females in the 1,000 mg per kg bw per day dose group.

The histopathology findings for the adrenals, parts of the small and large intestines, kidney, liver, and stomach were independently examined by Experimental Pathology Laboratories (EPL) to assess the adverse effects at the cellular level (EPL, 2012) and were summarized by Maronpot (2015). No toxicologically significant findings were identified in an examination of the adrenals, cecum, and stomach. The effects observed on the adrenal gland were reported to be frequently observed in rat toxicological studies (Laast et al., 2014). Small periportal cytoplasmic vacuoles in the liver are commonly observed in preclinical studies with rodents, and were not considered toxicologically significant. Changes in the diffuse hepatocyte cytoplasmic vacuoles were observed only in rats treated with Ashitaba Chalcone Powder and were attributed to intracytoplasmic triglycerides that result from changes in lipid metabolism and which are usually reversible. No hepatocellular degeneration or necrosis was observed, so the changes were not considered toxicologically significant. Renal data were considered to be consistent with spontaneous chronic progressive nephropathy (CPN), an age-related disease occurring in rats. The renal lesions were consistent with alpha 2-urinary globulin nephropathy. However, neither CPN nor alpha 2-urinary globulin nephropathy are relevant to human risk assessment; therefore, these findings are not toxicologically relevant to humans. In the jejunum, the dilated lacteals were determined to be an unusual finding in preclinical toxicity studies, as no chronic or granulomatous changes were noted. These adverse effects were noted in the jejunum at the 1,000 mg per kg bw per day dose level. Based on this observation and the pathology review by EPL, Maronpot determined the NOAEL for Ashitaba Chalcone Powder to be 300 mg per kg in male and female rats.

JBSL-USA concludes that the totality of evidence from these toxicity studies, including physical, hematological, and pathological results, supports the safety of Ashitaba Chalcone Powder (8%). Furthermore, Maronpot's pivotal acute toxicity and subchronic toxicity studies conducted with JBSL-USA's Ashitaba Chalcone Powder found the LD_{50} to be higher than 2,000 mg per kg bw in rats and the NOAEL to be 300 mg per kg bw per day in rats, respectively, served as the basis for this GRAS conclusion.

6.4.4 Reproductive and Developmental Toxicity Studies

No reproductive or developmental toxicity studies were identified in the published literature.

6.4.5 Cytotoxicity, Genotoxicity, and Mutagenicity Studies

Maronpot (2015) investigated the mutagenicity of Ashitaba Chalcone Powder (8% chalcone, JBSL-USA) in a bacterial reverse mutation assay. The study adhered to OECD Principles of GLP. The test

article was prepared by dissolving Ashitaba Chalcone Powder in dimethyl sulfoxide (DMSO) at a concentration of 125 mg per mL. Bacterial strains *S. typhimurium* TA98, TA100, TA1535, TA1537, and *E. coli* strain WP2 *uvr* A were used. The Ashitaba Chalcone Powder solution was added at concentrations of 12.3, 37, 111, 333, and 1,000 µg per plate. Compared with the negative control, the Ashitaba Chalcone Powder solution was cytotoxic for *S. typhimurium* strain TA100 in the presence and absence of metabolic activation. The Ashitaba Chalcone Powder solution did not elicit a two-fold or greater or a dose-related increase in the mean number of revertant colonies in any strains in either the presence or absence of metabolic activation. The mean number of *his and *trp revertant colonies of negative controls were within the acceptable range. The positive control groups had the expected significant increase in the number of reverse mutants in the presence and absence of metabolic activation. Maronpot concluded that Ashitaba Chalcone Powder is not mutagenic for the strains under the study conditions.

A pair of studies were conducted by Maronpot (2015) to investigate the ability of Ashitaba Chalcone Powder (8% chalcone, JBSL-USA) to induce structural chromosomal aberrations in Chinese hamster ovary (CHO) cells. The studies adhered to GLP and OECD Guideline 473. The final concentrations of Ashitaba Chalcone Powder in the culture medium ranged from 10 to 2,500 µg per mL. Tests were conducted in both the presence and absence of s9 metabolic activation. By the end of the four-hour treatment period, all cells in the 313, 625, 1,250, and 2,500 µg per mL Ashitaba Chalcone Powder treatment groups were dead; therefore, the concentrations were considered cytotoxic and were not used in subsequent studies.

In the first study, non-cytotoxic doses below 156 µg per mL were tested. In the absence of metabolic activation, treatment times were four and 18 hours, and in the presence of metabolic activation, treatment time was four hours. Cells were harvested at 18 hours after the onset of treatment. At the 156 µg per mL treatment level, the mitotic index was reduced to 66% after four-hours in the presence of metabolic activation, whereas lower doses (39 and 78 µg per mL) were not observed to effect mitoses compared to the vehicle control. In the absence of metabolic activation after the four-hour treatment period, all cells in the 156 µg per mL treatment group died prior to harvesting at 18 hours. Thus, the highest concentration analyzed was 78 µg per mL Ashitaba Chalcone Powder. At the 78 µg per mL treatment level, the mitotic index was 78% of the concurrent control in the absence of metabolic activation. There was no reduction to the mitotic index for the 20 and 39 µg per mL treatments compared with the concurrent control. There was no significant increase in the number of aberrant cells at any concentration of Ashitaba Chalcone Powder that was analyzed.

In the second study, the cyclophosphamine positive control did not yield expected results at the 32-hour sample timepoint; therefore, the assay was repeated. In the absence of metabolic activation, treatment times was 18 hours, with harvesting at 18 and 32 hours. In the presence of metabolic activation, treatment time was four hours, followed by harvesting at 18 and 32 hours. In the presence

of metabolic activation, the mitotic indices for the 200 µg per mL, 250 µg per mL, and 300 µg per mL treatment groups were 77%, 75%, and 78%, respectively. A significant increase in the number of aberrant cells treated with 300 µg per mL Ashitaba Chalcone Powder was observed, which the author attributed to the severe cytotoxicity of Ashitaba Chalcone Powder at this dose based on the first study. There was no significant increase in the number of aberrant cells in the absence of metabolic activation at any concentration of Ashitaba Chalcone Powder that was analyzed. Maronpot (2015) concluded that Ashitaba Chalcone Powder did not induce genotoxic effects and was cytotoxic, but not clastogenic, for CHO cells.

An *in vivo* micronucleus study was conducted to assess the ability of Ashitaba Chalcone Powder (7.2% chalcone, JBSL-USA) to induce micronuclei in the bone marrow of Swiss CD-1 mice (Maronpot, 2015). The study adhered to OECD Guideline 474 and GLP. A range-finding study was conducted on groups of three male and three female mice at doses of 500, 1,000, and 2,000 mg Ashitaba Chalcone Powder per kg bw. There were no observed differences in response based on sex; therefore, the main study was conducted using male mice (n=14 per group). Mice were treated with either 2,000 mg per kg Ashitaba Chalcone Powder, methylcellulose (vehicle control), or 70 mg cyclophosphamide per kg bw (positive control). At 24 and 36 hours post-treatment, seven mice in each treatment group were euthanized. There was no significant difference in the number of micronuclei in polychromatic erythrocytes in the bone marrow of mice in the ashitaba-treated group compared to the negative control group. The author concluded that Ashitaba Chalcone Powder is not genotoxic *in vivo*.

JBSL-USA concludes that the totality of evidence from these genotoxicity and mutagenicity studies supports the safety of Ashitaba Chalcone Powder (8%).

6.4.6 Carcinogenicity Studies

No carcinogenicity studies were identified in the published literature.

6.4.7 Published Animal Studies

A large number of scientific studies have been published in which ashitaba and ashitaba-derived preparations have been evaluated in laboratory animals. Any studies that were identified as particularly valuable to the safety assessment of Ashitaba Chalcone Powder (8%) are discussed in detail below; however, many studies did not monitor specific safety endpoints or report on adverse effects. Since the observed metabolism and/or health effects of these *in vivo* studies may reveal potential safety related concerns, a brief summary of studies is provided in Appendix 8.

Kimura and Baba (2003) reported that a 50% ethanol extract of *A. keiskei* roots and an ethyl-acetate-soluble fraction of the 50% ethanol extract reduced tumor volume and inhibited tumor metastasis compared to the control group when administered orally twice per day for 30 consecutive days at 500 mg per kg bw or 200 mg per kg bw, respectively, starting 12 hours after implantation of tumor cells in

female C527Bl/6 mice. Neither the ethanol extract nor the ethyl acetate-soluble fraction had a significant effect on the weights of the spleen or thymus.

The ethyl acetate-soluble fraction was further separated into two fractions, Fraction 1 and Fraction 2. Both fractions, when administered orally at a dose of 100 mg per kg bw twice daily for 14 consecutive days, inhibited tumor growth and metastasis to the lung. An increase in lung weight was also observed. There were no changes to the spleen weight. An increased survival rate was observed in mice from which the tumor was removed. XA was isolated from Fraction 2, and was administered at 50 or 100 mg per kg bw per day for 14 days, beginning 12 hours after transplantation of tumor cells into the right abdomen of mice. On day 15, tumor volume was measured and tumor tissues were resected. Beginning 23 hours after the resection of the tumor, XA was administered orally at 25 or 50 mg per kg bw twice per day, and the survival rate was monitored. Animals that had not already died were euthanized on day 18. Oral administration of XA at 50 mg per kg bw twice per day was observed to inhibit primary tumor growth and metastasis to the lung, reduce lung weight, prolong survival rate, and increase the survival rate of the carcinectomized mice compared with tumor-bearing mice. Administration of 25 mg XA per kg bw twice daily reduced tumor weight, but had no effect on lung weight. The authors stated that XA "did not cause apparent adverse reactions" (Kimura and Baba, 2003).

In a follow-up study, Kimura et al. (2004) administered 4-HD, isolated from *A. keiskei* roots, to six-week-old Lewis lung carcinoma (LLC)-bearing C57BL/6J female mice at doses of 25 or 50 mg per kg bw twice daily for 14 days beginning 12 hours after implantation of the tumor cells. A group of mice were treated with 5 mg per kg doxorubicin via i.p. injection on days 1 and 8. Tumor volume was measured every two to three days. All LLC tumor-bearing mice lived to day 15. Tumors were removed from mice on day 15 and weighed. Beginning 24 hours after tumor resection, mice were administered the same treatment they received prior to surgery until they died. A group of sham operated mice and mice whose tumors were not removed were given water alone according to the schedule above. Surviving animals were euthanized on day 16. Oral administration of 4-HD at a dose of 25 or 50 mg per kg bw twice per day resulted in a significant inhibition of tumor growth from day 8 to day 14. The administration of 50 mg 4-HD per kg bw twice daily significantly inhibited the tumor weight on day 15. Treatment with 4-HD prolonged survival times and increased survival rate compared to the tumor-removed mice. In addition, treatment with 4-HD inhibited lung metastasis after removal of subcutaneous tumors and the elevation of lung and spleen weight. A reduction in thymus weight in 4-HD-treated mice from which the tumor was removed was also observed. For mice with intrasplenically implanted LLC cells, oral administration of 50 or 100 mg 4-HD per kg bw per day for 19 days beginning at 12 hours after tumor implantation decreased the weight of the tumor in the spleen, prevented tumor growth and invasion of the white pulp of the spleen, induced surrounding of the tumor cells in the white pulp of the spleen with lymphocytes, and inhibited growth of metastatic tumors in the liver. When administered orally at a dose of 50 mg 4-HD per kg bw twice daily, CD4+, CD8+, and natural killer cells were inhibited in the spleens of tumor-removed mice. At a dose of 100 μM, 4-HD inhibited

DNA synthesis in LLC cells, but elicited no change in DNA synthesis in human umbilical vein endothelial cells. At doses ranging from 10 to 100 µM, 4-HD inhibited formation of capillary-like tubes by human umbilical cord vein endothelial cells cultured in Matrigel. No adverse effects of 4-HD administration were reported.

The effect of dietary *A. keiskei* stem juice extract on lipid metabolism in rats was investigated in a series of studies conducted by Ogawa et al. Ashitaba stem juice extract was prepared from an ethyl acetate and distilled water (4:1, v/v) extraction of *A. keiskei* stem juice. Six-week-old male stroke-prone spontaneously hypertensive (SHRSP) rats were fed a control diet (n=6) or 0.2% ashitaba stem juice extract added to the control diet (n=6) for six weeks (Ogawa et al., 2003). Food and water were offered *ad libitum.* Body weight, average food intake, blood pressure, serum enzyme activity, total liver lipids, cholesterol, phospholipid, triglyceride contents, and total RNA from the liver were measured. There was no significant effect of *A. keiskei* stem juice extract on blood pressure. No significant differences were observed in growth rate and food intake between the two treatment groups. Furthermore, there were no significant differences in the treatment groups with respect to glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, alkaline phosphatase, triglycerides or apolipoprotein B. Cholesterol, phospholipids, apolipoprotein A-I and apolipoprotein E were significantly higher in the ashitaba stem juice extract group than the control group. The relative liver weight and triglycerides in the liver were significantly lower in the ashitaba stem juice extract group compared with the control group. Ogawa et al. (2003) concluded that ashitaba stem juice extract may enhance reverse cholesterol transport and inhibit hepatic lipid accumulation. No adverse effects were reported.

For subsequent experiments, laserpitin, 4-HD, and XA were purified from the ashitaba stem juice ethyl acetate extract. Six-week-old male SHRSP rats were fed a diet containing the isolated compound for seven weeks (n=six rats per dose group for all the studies). Dietary intake of 0.1% laserpitin (Ogawa et al., 2005a) resulted in a reduction in body weight gain after week four through week seven compared to the control diet (no laserpitin) group, but there was no difference in food intake between groups. Serum cholesterol, phospholipid, and apolipoprotein E (apo E) levels were significantly increased in the laserpitin group, which was due to significant increases in cholesterol, phospholipids, and apo E contents of the LDL and HDL fractions. No significant changes in apo A-1 and apo B were observed in the serum. Blood pressure was unaffected by laserpitin consumption. There were no differences in the serum concentrations of triglyceride and free fatty acids, apo B, and the apo A/apo B ratio between groups. Significant decreases in serum glucose level, relative liver weight, and triglyceride content of the liver were also observed. No adverse effects were reported.

Elevation of systolic blood pressure was significantly suppressed in male SHRSP rats treated with 0.07% 4-HD in the diet after four to seven weeks of treatment (Ogawa et al., 2005b). Serum levels of cholesterol, phospholipids, and triglycerides were significantly reduced in the VLDL fraction without concurrent effect on HDL levels and were associated with significant decrease in the serum

concentration of fatty acids. Significant decreases in relative liver weight and triglyceride content of the liver were also observed. There were no changes in body weight, growth rate, food intake, serum cholesterol, serum phospholipid, and triglyceride. There were no changes in liver content of apo A-I, apo B, apo E, or cholesterol. No adverse effects were reported.

Six-week-old male SHRSP rats were fed diets containing 0.02% or 0.1% XA in the diet for seven weeks (Ogawa et al., 2007). There were no significant changes in food intake, body weight, or systolic blood pressure over the course of the study. Significant dose-dependent decreases in relative liver weight and total triglyceride content were observed in both XA treatment groups. In the 0.1% XA treatment group, a significant decrease in total cholesterol in the liver was observed. Dose-dependent reductions in cholesterol and phospholipid contents in the LDL fraction were reported. No significant changes in serum concentrations of cholesterol, phospholipid, triglyceride, free fatty acids, glucose, and apo A-I, apo B, apo E, or the apo B/apo A-I ratio were observed among the three groups. No adverse effects were reported.

Enoki et al. (2007) studied the effect of ashitaba extract on five-week-old male KK-A^y/Ta mice, which develop diabetes and hyperglycemia with aging. Mice were divided into three groups of 7-10 animals based on body weight and blood glucose level. Ashitaba extract was prepared by extracting dried, powdered ashitaba roots with 100% ethanol, from which 4-HD and XA were purified. Mice were fed a powdered diet containing 0.15% 4-HD or XA, or the control diet alone. Food and water were provided *ad libitum* for four weeks. Body weight, food intake, and water intake were measured and blood samples were collected from the tail vein for blood glucose levels. After two weeks, there were 50% and 33% reductions in the elevation of blood glucose levels by ingestion of 4-HD and XA, respectively.

A subsequent seven-week study was performed to investigate the long-term effects of 4-HD intake on hyperglycemia, polydipsia, and body weight (Enoki et al., 2007). The same animal model was used, and pioglitazone (0.05% in the diet) was also tested for the ability to prevent the progression of diabetes. The elevation of blood glucose levels was suppressed in the 4-HD group compared to the control. Furthermore, 4-HD administration inhibited polydipsia. Both polydipsia and hyperglycemia were almost completely suppressed in mice fed the experimental diet containing 0.05% pioglitazone. An increase in body weight was observed in mice fed the diet containing pioglitazone, while mice in the 4-HD group displayed body weights similar to the control group. The authors noted that "no obvious toxicities or any other adverse effects" were observed in the 4-HD and XA treatment groups.

In a follow-up study, Enoki et al. (2010) evaluated the anti-diabetic effect of 4-HD on blood glucose levels in hyperglycemic mice. The 4-HD test material was purified from an ethanolic extract of dried, powdered ashitaba roots. Twelve-week-old male KK-A^y/Ta mice were divided into two treatment groups: 200 mg 4-HD per kw bw per day in 0.5% carboxymethylcellulose (n= 5) or carboxymethylcellulose (n=5) for 18 days via gavage. Blood sugar levels were monitored in blood collected from the caudal vein. Reduced blood glucose levels were observed on days 4 and 18 for mice in the 4-HD group compared with the control; however, there was no significant difference between

groups on day 11. No significant differences in body weight or general health of the mice were observed between groups, and no adverse effects were observed during pathological analyses of the organs at the conclusion of the study.

A study performed by Ohnogi et al. (2012a) examined the effect of an ethanol extract of *A. keiskei* on insulin resistance and hypertriglyceridemia in fructose-drinking rats as a model for metabolic syndrome. Dried ashitaba leaves and stems were washed with hot water, and the washed residue was extracted with 90% ethanol prior to concentration and drying. The resulting ashitaba extract contained 7.30 g XA per kg and 3.45 g 4-HD per kg, which accounted for 97% of the total chalcones. Six-week-old male Wistar rats were separated by body weight into three groups (n= eight rats per group). The fructose group and the fructose-ashitaba extract group were given 15% fructose solutions as drinking water and fed a powdered CE-2 diet, with and without 3% (w/w) ashitaba extract, respectively, for 11 weeks. Rats in the normal group were fed the powdered diet and water. There were no significant differences in body weight among the groups at the end of the experiment, although the fructose and fructose-ashitaba extract groups had reduced daily food consumption and increased daily fluid intake compared with the normal group. There was no difference in liver triglycerides among the groups. Rats in the fructose group had significantly higher absolute and relative liver weights than those in the normal group. Addition of ashitaba extract to the diet significantly reduced the absolute and relative liver weights compared with the fructose diet group. Furthermore, treatment with ashitaba extract significantly reduced the levels of blood glucose, serum insulin, homeostasis model assessment insulin resistance (HOMA-R), serum triglycerides, and free fatty acids compared with rats in the fructose-only treatment group; however, a significant increase in total cholesterol and HDL was observed in the ashitaba extract treatment group compared to the fructose-only and normal treatment groups. Ohnogi et al. (2012a) concluded that ashitaba extract inhibited insulin resistance and hyperglycemia in rats that consumed fructose. No adverse effects were reported.

Zhang et al. (2015) investigated the effect of an ethyl acetate extract of ashitaba on adiposity in mice fed a high fat diet. Ashitaba extract containing 64.89 mg per g 4-HD and 84.86 mg per g XA was prepared from an ethyl acetate extract of Ashitaba Chalcone Powder (JBSL). Six-week-old male C57BL/6 mice were randomly divided into six group of six mice each and fed either the control diet or high fat diet containing 30% (w/w) lard for 16 weeks. The diets were supplemented with 0%, 0.01%, or 0.1% ashitaba extract. After 16 weeks, mice were fasted for 18 hours and euthanized.

Body weights of the mice fed the high fat diet supplemented with 0.01% and 0.1% ashitaba extract were significantly lower than the high-fat control mice from weeks 10 to 16. Supplementation with ashitaba extract suppressed the high fat diet increased adipose tissue weight compared to the high fat control, but did not affect the body weight or adipose tissue weight of the mice fed the control diet. Total plasma cholesterol was significantly increased in the high fat control group compared with the control diet group, whereas supplementation with ashitaba lowered the plasma cholesterol level to

almost the same level as the control diet mice. Ashitaba did not affect plasma triglyceride and non-esterified fatty acid (NEFA) levels in the control or high fat diet groups. High fat diet mice supplemented with ashitaba extract displayed significantly lower hepatic total lipid, triglyceride, and cholesterol levels than the high fat control mice, while ashitaba extract had no effect on the same parameters in control diet mice. Plasma glucose, insulin, and HOMA-IR were significantly higher in the high fat diet group compared to the control diet group; however, supplementation with ashitaba extract in the high fat diet reduced all three parameters. No effect on plasma glucose, insulin, or HOMA-IR was observed in mice fed the control diet. Furthermore, ashitaba extract ameliorated the decreased adiponectin level in the high fat diet groups in a dose-dependent manner, where the 0.1% treatment group had a similar adiponectin level as the control diet groups. No unscheduled animal deaths or adverse effects were reported.

Li et al. (2016) studied the effect of XA on inflammation in high-fat diet-induced obese mice. Six-week-old male C57BL/6J mice were fed a diet of commercial chow (CFR-1, control); a high-fat diet; or a high-fat diet supplemented with 0.1 or 0.15% (w/w) XA for 14 weeks. The plasma concentration of XA increased in a dose-dependent manner. Mice in the 0.15% XA supplementation group exhibited significantly reduced body weight, adipose tissue mass, and liver triglyceride levels compared with the high-fat diet group. No differences in food intake between the groups were observed. No adverse effects were reported.

6.4.8 Summary of Animal Studies

JBSL-USA concludes that the totality of evidence from these animal studies, including physical, hematological, and pathological results, in addition to the absence of reported adverse effects, supports the safety of Ashitaba Chalcone Powder (8%). Furthermore, as previously stated, Maronpot's pivotal acute toxicity and subchronic toxicity studies -- conducted with JBSL-USA's Ashitaba Chalcone Powder -- found the LD_{50} to be higher than 2,000 mg per kg bw in rats and the NOAEL to be 300 mg per kg bw per day in rats, respectively, served as the basis for this GRAS conclusion.

6.4.9 Human Studies

Ohnogi et al. (2012b) examined the effect of ashitaba green juice treatment in an open label pilot study. Ashitaba granules were prepared from dried leaves and stems that were powered and spray dried with dextrin, with 198.7 mg chalcones per 100 g granules (134.5 mg XA and 64.2 mg 4-HD per 100 g granules, respectively). Nine Japanese adult subjects (seven male and two female) were selected for the study based on one or more of the following criteria: waist circumference > 85 cm (males) and > 90 cm for females, ≥ 110 mg per dL fasting plasma glucose, ≥ 150 mg per dL triglycerides, ≥ 130/85 mmHg blood pressure, or < 40 mg per dL HDL-cholesterol. Study participants, aged 35 to 60 years, were given 6.2 g of granulated ashitaba (12.3 mg chalcones) twice a day with water, at breakfast and at supper, for eight weeks. None of the study participates were taking any medicine, had serious health complaints, or had been diagnosed with a serious disease. Blood and urine samples were collected at four-week intervals. Occult blood was observed in one male study participant in week

four, but the authors did not consider it to be related to ashitaba ingestion. No severe symptoms were observed during the study and adverse events included nasal mucus (n = one), sore throat (n = two), and toothache (n = one). All subjects completed the study. Ohnogi et al. (2012b) concluded that no serious clinical symptoms occurred and that consumption of ashitaba is safe.

Ohnogi et al. (2012c) evaluated the safety of ashitaba green juice powder (Takara Bio Inc., Japan) in an open-label study on healthy and borderline mildly diabetic subjects. Ashitaba green juice powder was prepared by drying, pulverizing, and sterilizing ashitaba granules with 5% dextrin. The chalcone content (as XA and 4-HD) was 192. 1 mg per 100 g ashitaba green juice powder. Twenty-five subjects with fasting blood sugar levels < 140 mg per dL were supplemented with 31.5 g ashitaba green juice powder per day for 4 weeks. This level of consumption was 5-10X greater than the "standard amount" of 3 to 6 grams ashitaba green juice powder. Study participants were not allowed to consume drinks and foods that have similar effects as ashitaba green juice powder. Blood tests and pathological examinations were conducted at the beginning of the intervention period, at the end of the second and fourth weeks, and at the end of a two-week-long post-observation period that began immediately after the end of the fourth week of supplementation. Urinalysis occurred at the beginning of intervention, at the end of the fourth week of supplementation, and during the post-observation period. Physical tests, blood chemistry analysis, hematologic assessment, and an adverse effect interview with a doctor were conducted.

Twenty-four subjects (11 men and 13 women, mean age = 51.75 ± 9.34 years of age) completed the study. One participant dropped out for unspecified reasons. A significant elevation in pulse rate was observed at the end of the fourth week and at the end of the post-observation period. These were not considered clinical abnormalities because the values remained within normal physiological range. Ohnogi et al. (2012c) noted that although significant hematological changes were observed during the study period compared to baseline for several subjects, no clinical abnormality was confirmed.

Adverse reactions in the treatment group included hives and a sense of bloating in the lips and mouth (n=1), stomatitis (n=1), and loose stool and/or a difficulty in defecating (n=4). The subject who developed hives on the eighth day of the study discontinued the ashitaba green juice powder on day 13, took medication, and recovered. The cause for hives could not be specified because there was no abnormality observed in the blood test and urinalysis and the participant had never consumed green juice including ashitaba prior to the study. A test for allergy to other umbelliferous vegetables, including parsley and celery, was negative. A doctor concluded that although it was possible that there could have been an allergic response to the test article, the likelihood was low. Soft stools and a sense of difficulty in defecating were thought to be possibly related to the consumption of the test article, but were not considered clinically abnormal because symptoms were mild. Stomatitis occurred in one individual beginning in the fourth week of the study, lasted for five days, and resolved on its own. Because the symptoms were mild, temporary, and began after nearly four weeks of supplementation, the relationship between consumption of ashitaba green juice powder and

development of stomatitis was conserved by the authors to be low. Ohnogi et al. (2012c) concluded that there are no safety concerns associated with this level of intake of ashitaba green juice powder.

A two-part randomized, placebo controlled, double blind study was conducted to determine the effect of JBSL-USA's Ashitaba Chalcone Powder on body weight and visceral fat in slightly obese adults (Ohnishi and Hackel, 2017). The study protocol was approved by the Akihabara Medical Clinic Ethics Committee and was conducted in compliance with the Declaration of Helsinki. Twenty-six overweight (25 <BMI< 30) male and female adults aged 40-65 years old were randomly assigned into two groups of 13 subjects (9 males and 4 females per group). Subjects received either placebo (control) or 200 mg per day Ashitaba Chalcone Powder after dinner for 56 days. No significant changes were observed within or between groups with regard to safety parameters, and no serious adverse events or medically significant changes were observed. Furthermore, Ohnishi and Hackel (2017) concluded that results of this two-part study support the safety of 200 mg per day Ashitaba Chalcone Powder (equivalent to ~16 mg per day total chalcones).

A randomized, double-blind, placebo-controlled pilot study was conducted to evaluate the effects of JBSL-USA's Ashitaba Chalcone Powder on metabolic health parameters in subjects with metabolic syndrome (Kalman et al., 2018). Subjects were non-smokers aged 35 to 70 years of age. Inclusion criteria included a waist circumference ≥40 inches for males and ≥35 inches for females, a BMI of 25.0 to 40.0 kg per m^2, and the presence of one of the following indicators of metabolic syndrome: raised blood pressure (120 to 160 mmHg systolic and 80 to 90 mmHg diastolic); > 150 mg per dL triglycerides; < 40 mg per DL and < 50 mg per dL HDL for men and women, respectively; ≥ 100 mg per dL fasting glucose; and 5.7 to 7.0% HgA1c. Sixty adult subjects were randomly assigned to two groups of 15 men and 15 women, each. Subjects received either a placebo (control) or 220 mg per day Ashitaba Chalcone Powder (standardized to 3% 4-HD and 5% XA) for 12 weeks. One male subject in the placebo group dropped out of the study after visit 2 (cause not reported). No significant changes were reported within or between groups with regard to safety parameters. No adverse effects were reported.

A significant increase in systolic blood pressure has not previously been reported in human clinical studies. Ohnogi et al. (2012b) observed no changes in blood pressure in subjects treated with ashitaba green juice prepared from the leaves and stems of *A. keiskei.* Ohnishi and Hackel (2017) monitored blood pressure as a safety parameter in an 8-week study on ashitaba chalcone supplementation, and reported that no significant changes were observed in safety parameters throughout the study. Given the relatively small sample size (four subjects in the test group) of the Fuentebella et al. (2019) study, it is possible that the observed increase in systolic blood pressure is an anomaly.

Rahmi et al. (2020) investigated the effect of *A. keiskei* extract in concurrence with nifedipine treatment on the blood pressure of 30 post-partum women with hypertension. The study protocol was not well-described by the authors, as the nature of the ashitaba extract, treatment doses, and

duration of the study were not reported. While the study is of limited value in terms of assessing safety parameters, it should be noted that no adverse effects were reported.

Additional studies have been performed on commercially-available green vegetable juice preparations in which ashitaba is an ingredient. These studies provide limited relevant safety information, since it is impossible to attribute any effects, adverse or otherwise, to *A. keiskei*. Therefore, a table summarizing human studies on ashitaba-containing green juice mixtures is provided in Appendix 9. No potential safety-related concerns were noted.

6.4.10 Summary of Human Studies

JBSL-USA concludes that the totality of evidence from these human clinical studies supports the safety of Ashitaba Chalcone Powder at consumption levels of up to 157 mg per day.

6.4.11 Biological Activity

The biological activity and mechanisms of action of an ingredient or components of an ingredient may reveal potential safety related concerns. Therefore, a table summarizing of the studies on biological activity of *A. keiskei* and preparations derived from *A. keiskei* is provided in Appendix 10. No potential safety-related concerns were noted.

6.4.12 Overall Summary of Published Safety Studies

The primary evidence of safety for JBSL-USA's Ashitaba Chalcone Powder (8%) comes from published toxicological and clinical studies on JBSL-USA's Ashitaba Chalcone Powder and related *A. keiskei* preparations. Primary findings from the pivotal safety study by Maronpot (2015) include:

- Ashitaba Chalcone Powder (8% chalcone, JBSL-USA) showed no acute oral toxicity in rats after a single dose of 2,000 mg per kg bw. Maronpot (2015) concluded the LD_{50} was great than 2,000 mg per kg bw since no mortality was observed at this dose level.
- Ashitaba Chalcone Powder (8.45% chalcone, JBSL-USA) showed no subchronic toxicity when administered orally to rats in a 13-week study at doses up to 300 mg per kg bw per day. A NOAEL of 300 mg per kg bw per day was reported (Maronpot, 2015).
- No significant toxicity was observed when a dose of 600 mg per kg bw Ashitaba Chalcone Powder (JBSL-USA) was orally administered to rats in a 90-day study, supporting the NOAEL of 300 mg per kg bw per day, as reported by Maronpot (2015).
- No mutagenic effects were observed in multiple bacterial reverse mutation assays performed on methanolic and ethanolic ashitaba extracts or ashitaba chalcone powder preparations (Kwon et al., 2006; Ohnogi et al., 2012d; Maronpot, 2015).
- There are no known reports of allergenicity for JBSL-USA's Ashitaba Chalcone Powder (8%).

JBSL-USA weighed the results of the subchronic toxicity studies more heavily than other rodent studies or *in vitro* studies. Using the NOAEL from Maronpot (2015) of 300 mg Ashitaba Chalcone

Powder per kg bw per day and a safety factor of 100, the ADI for Ashitaba Chalcone Powder in humans can be calculated as 3 mg Ashitaba Chalcone Powder per kg bw per day or 210 mg Ashitaba Chalcone Powder per person per day, using 70 kg as the reference body weight for humans. The Ashitaba Chalcone Powder in the study was reported to contain 8.45% chalcones; therefore, the ADI can be extrapolated to be equivalent to 17.7 mg chalcones per person per day.

The highly conservative 'mean X 2' EDI of 31.8 mg Ashitaba Chalcone Powder per person per day is expected to be significantly lower than the calculated ADI of 210 mg Ashitaba Chalcone Powder per person per day.

Although JBSL-USA placed emphasis on studies conducted on ashitaba chalcone preparations, a number of studies have been performed of various extracts or other preparations derived from the leaves, root, or aerial parts of *A. keiskei*. Many of these preparations contain different concentrations of the chalcones, flavones, coumarins, and other compounds found in ashitaba yellow sap. Therefore, these studies provide supportive evidence for the safety of JBSL-USA's Ashitaba Chalcone Powder (8%). Key findings include:

- Ashitaba powder (plant part unknown) showed no acute oral toxicity in rats after a single dose of 3,500 mg per kg bw ashitaba powder. A NOAEL of 3,500 mg per kg bw was reported (Ohnogi et al., 2012d).
- Ashitaba powder (plant part unknown) showed no significant subchronic toxicity when administered to rats orally for 13 weeks at doses up to 1,750 mg per kg bw per day. A NOAEL of 1,750 mg per kg bw per day was reported (Ohnogi et al., 2012d).
- Animal studies that investigated the effects of ashitaba powder on serum and lipid profiles and body fat accumulation (Nagata et al., 2007; Kim et al., 2012a) and hyperlipidemia and hepatic steatosis (Kwon et al., 2018) reported no adverse effects or significant safety concerns raised by pathological, biochemical, and physical observations.
- No safety concerns or serious clinical symptoms were reported in two open label studies on ashitaba green juice, with intakes of 6.2 g granulated ashitaba (corresponding to 12.3 mg chalcones) twice per day for eight weeks or 31.5 g ashitaba green juice (corresponding to ~60 mg chalcones) per day for four weeks. Adverse effects were considered mild and included nasal mucus, sore throat, toothache, hives, stomatitis, loose stool and/or difficulty defecating (Ohnogi et al., 2012b; c).
- Fuentebella et al. (2019) reported a significant increase in systolic blood pressure in subjects with type II diabetes mellitus following supplementation with 1,500 mg per day ashitaba for two weeks. An increase in systolic blood pressure was not observed in other published clinical studies by Ohnogi et al. (2012c) and Ohnishi and Hackel (2017).
- No adverse effects or safety concerns were reported by Oh et al. (2019) in a clinical study on juice obtained from *A. keiskei* leaves.

JBSL-USA's Ashitaba Chalcone Powder (8%) is manufactured under CGMP practices from the yellow sap exudate of *A. keiskei*. Botanical identity is confirmed by microscopy and HP-TLC. Appropriate limits have been established for moisture content, heavy metals, and microbial contaminants. JBSL-USA's Ashitaba Chalcone Powder (8%) consistently meets these specifications, as demonstrated in data provided for five representative, nonconsecutive lots of material. Furthermore, representative pesticides analyses have shown that there are no detectible residues in the material.

Based on the evidence of safety described herein, JBSL-USA concludes there are no safety concerns with consumption of Ashitaba Chalcone Powder (8%) as an ingredient in conventional foods. While there is limited documentation on history of use in the United States, ashitaba leaves and ashitaba leaf juice are commonly consumed as food in Japan and Korea. Based on proposed uses in soft drinks, fruit preserves, candy containing chocolate, and butter, the mean total EDI for all individuals was determined to be 15.9 mg Ashitaba Chalcone Powder (8%), and the highly conservative 'mean X 2' was determined to be 31.8 mg Ashitaba Chalcone Powder (8%), with a total maximum consumption estimate of 157 mg Ashitaba Chalcone Powder (8%). These estimated intakes fall well below the calculated ADI of 210 mg per person per day and therefore there is a high presumption of safety from the proposed use of Ashitaba Chalcone Powder (8%) as a conventional food ingredient.

Furthermore, a number of generally available, relevant toxicity studies, ADME studies, and clinical studies were found in the published literature to support the conclusion that Ashitaba Chalcone Powder (8%) is well-tolerated in humans. JBSL-USA asserts that well-qualified scientists would conclude that Ashitaba Chalcone Powder (8%) is GRAS based upon available common knowledge and scientific procedures.

6.5 Other Safety Considerations

6.5.1 Allergenicity

A search of PubMed[11] for the terms "ashitaba allergy," "ashitaba hypersensitivity," "Angelica keiskei allergy," and "Angelica keiskei hypersensitivity" yielded no results. There are no known reports of allergenicity to JBSL-USA's Ashitaba Chalcone Powder (8%) or any other *A. keiskei* preparations.

6.5.2 Antinutritional Factors

A search of PubMed for the terms "ashitaba antinutritional factor" and "Angelica keiskei antinutritional factor" yielded no results.

[11] Available at: https://www.ncbi.nlm.nih.gov/pubmed (Accessed on May 25, 2021)

6.6 Conclusions

Based on the composite data and information presented herein, JBSL-USA has concluded that Ashitaba Chalcone Powder (8%), meeting appropriate food grade specifications and manufactured according to CGMP, is GRAS as an ingredient in conventional foods on the basis of scientific procedures, in accordance with FDA's standard for food ingredient safety, i.e., reasonable certainty of no harm under the intended conditions of use.

Part 7. List of Supporting Data and Information

Abbreviations

4-HD	4-Hydroxyderricin
ABTS	2,2'-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid)
ADI	Acceptable Daily Intake
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AOAC	Association of Official Analytical Chemists
apo E	Apolipoprotein E
AST	Aspartate aminotransferase
BAM	Bacteriological Analytical Manual
Bq	Becquerel
BUN	Blood urea nitrogen
bw	Body weight
CCl_4	Carbon tetrachloride
CFU	Colony forming unit
CGMP	Current Good Manufacturing Practices
CHO	Chinese hamster ovary
COAs	Certificates of Analysis
CPN	Chronic progressive nephropathy
DBH	Dopamine B-hydroxylase

DHO	(8S-9R)-8-angeloxy-8,9-dihydrooroselol
DMBA	7,12-dimethylbenz[*a*]anthracene
DMSO	Dimethyl sulfoxide
DNFB	1-Fluoro-2,4-dinitrobenzene
DPPH	1,1-diphenyl-2-picryl-hydrazl
EDI	Estimated Daily Intake
EFSA	European Food Safety Authority
EPL	Experimental Pathology Laboratories
FD&C Act	Food, Drug, and Cosmetic Act
FDA	Food and Drug Administration
FEMA	Flavor and Extract Manufacturers Association
FOIA	Freedom of Information Act
FSA	Food Standards Agency
FSANZ	Food Standards Australia New Zealand
FSIS	Food Safety and Inspection Service
g	Gram
GalN	D-galactosamine
GLP	Good laboratory practice
GRAS	Generally Recognized as Safe
GRIN	Germplasm Resources Information Network
HDL	High-density lipoprotein
HOMA-R	Homeostasis model assessment insulin resistance
HP-TLC	High performance thin-layer chromatography
HPLC	High performance liquid chromatography
i.p.	Intraperitoneal
IC_{50}	Half maximal inhibitory concentration
ICP/MS	Inductively coupled plasma/mass spectrometry
ILA	Isolaserpitin
ISO	International Organization for Standardization
JECFA	Joint FAO/WHO Expert Committee on Food Additives

JMHLW	Japanse Ministry of Health, Labour and Welfare
LAS	Laserpitin
LD_{50}	Median lethal dose
LDL	Low-density lipoprotein
LLC	Lewis lung carcinoma
LPS	Lipopolysaccharides
MAO	Monoamine oxidase
mg	Milligram
ND	Not detected
NEFA	Non-esterified fatty acid
NF-KB	Nuclear factor-KB
NHANES	National Health and Nutrition Examination Survey
NHC	National Health Commission of the People's Republic of China
NLT	Not less than
NMT	Not more than
NO	Nitric oxide
non-GMO	non-Genetically Modified Organism
OECD	Organisation for Economic Co-operation and Development
OH	Hydroxide
PAI-1	Plasminogen activator inhibitor-1
ppm	Parts per million
RACC	Reference amount customarily consumed
SHRSP	Stroke-prone spontaneously hypertensive rats
TNF-a	Tumor necrosis factor alpha
TPA	12-*O*-tetradecanoylphorbol-13-acetate
USDA	United States Department of Agriculture
VLDL	Very-low density lipoprotein
WHO	World Health Organization
XA	Xanthoangelol

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Appendix 1. Raw Material Organic Certification Documentation and ISO 9001:2015 Certification Documentation

OMIC

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CERTIFICATE

JAS OMIC

This is, in accordance with Act on Japanese Agricultural Standards (Law No. 175 of 1950), to certify Organic operation of PT. AMBITIOUS TRADING COY, LTD. with details as below:

OMIC Certification Number	:	No. 1297
Date of Certification	:	June 20, 2006
Party Certified	:	PT. AMBITIOUS TRADING COY, LTD. Jl. Dinoyo 29 Surabaya, Indonesia
AREA PANJERAN	:	Dusun Kemloko Desa Trawas Kecamatan Trawas Kabupaten Mojokerto, Indonesia
AREA CEMBOR	:	Dusun Cembor Desa Cembor Kecamatan Pacet Kabupaten Mojokerto, Indonesia
AREA TAPAN-1	:	Dusun Ketapanrame Desa Ketapanrame Kecamatan Trawas Kabupaten Mojokerto, Indonesia
AREA TAPAN-2	:	Dusun Ketapanrame Desa Ketapanrame Kecamatan Trawas Kabupaten Mojokerto, Indonesia
JAS Business Category	:	Overseas Production Process Manager
Kind of JAS Products	:	Organic Agricultural Products
Products	:	Organic Ashitaba, Organic Chalcone, Organic Konjac
Area	:	11,522.00 a
Parties involved in	:	PT. AMBITIOUS TRADING COY, LTD. (FACTORY) Desa Carat, Kecamatan Gempol, Kabupaten Pasuruan, Indonesia (Details as per attached sheet)

Overseas Merchandise Inspection Co., Ltd.
15-6, Nihonbashi Kabuto-cho, Chuo-ku,
Tokyo, Japan

[redacted]

Kiyoshi Cho, President

Issued Date : June 30, 2006
Revised Date : September 20, 2019

・Refer to the attachment for the list of "Appointed Persons in Charge".
・This certificate is to certify the production method of the Certified Business Entity in accordance with "Act on Japanese Agricultural Standards" (Law No.175 in 1950), but not to assure the quality or ingredients etc. of the products.

OVERSEAS MERCHANDISE INSPECTION CO., LTD.

Attached to OMIC Certification No. 1297

Appointed person in charge :

Production Process Management Director

Full Name
Mr. Tramiaji

Grading Manager

Full Name
Mr. Johan Soedjamiko

Grading Staff

Full Name
Mr. Winardi

Issued Date : June 30, 2006
Revised Date : September 20, 2019

OVERSEAS MERCHANDISE INSPECTION CO., LTD.

Attached Table to OMIC Certification No. 1297

Detail of Certified Farms

Producer		Address of Organic Certified Farm	Block No.	Area (a)	Kind of Certified Crop
PT. AMBITIOUS TRADING COY, LTD.	Mr. Tamiaji (Production Process Management Director)	Dusun Kemloko Desa Trawas Kecamatan Trawas Kabupaten Mojokerto, Indonesia	AREA PANJERAN	2,126.00	Ashitaba, Chalcone
		Dusun Cembor Desa Cembor Kecamatan Pacet Kabupaten Mojokerto, Indonesia	AREA CEMBOR	523.00	
		Dusun Ketapanrame Desa Ketapanrame Kecamatan Trawas Kabupaten Mojokerto, Indonesia	AREA TAPAN-1	776.00	
		Dusun Ketapanrame Desa Ketapanrame Kecamatan Trawas Kabupaten Mojokerto, Indonesia	AREA TAPAN-2	8,097.00	Ashitaba, Chalcone, Konjac
Total				11,522.00	

Issued Date : June 30, 2006
Revised Date : September 20, 2019

051 CM021

Japan Bio Science Laboratory Co., Ltd.

Head Office, Tokyo Branch, Kyushu Factory

1-4-40, Fukushima, Fukushima-ku, Osaka-city, Osaka, Japan

CERTIFICATE

Certificate No.: QC04J0134

ISO 9001:2015 · JIS Q 9001:2015

Development and manufacture of fermented soybean extract, fermented vegetable extract, vegetable-origin food materials and their products

Our organization certifies above organization to be complied with the requirement of indicated above management system.

Registration Date :14/Oct/2004
Recertification Date:14/Oct/2019
Issue Date :25/Sep/2019
Certificate Expiry :13/Oct/2022

Japan Audit and Certification Organization for Environment and Quality
2-2-19 Akasaka, Minato-ku, Tokyo, Japan

President & CEO

To be used in conjunction with attached Appendix

Appendix 2. ISOELEAT Supporting Documentation

Japan Bio Science Laboratory Co., Ltd.
URL : http://www.jbsl-net.com E-mail : kaihatu@jbsl-net.com
■OSAKA HEAD OFFICE
1-4-40 Fukushima, Fukushima-ku, Osaka-city, Osaka 553 0003 JAPAN
PHONE : +81-6-6451-1711 FAX : +81-6-6451-1712
■TOKYO OFFICE
12-2 Kandahigashimatsushita-cho, Chiyoda-ku, Tokyo 101-0042 JAPAN
PHONE : +81-3-5209-1601 FAX : +81-3-5209-1602
■FACTORY & LABORATORY
2022 Shioya, Akimachi, Kunisaki-city, Oita 873-0212 JAPAN
PHONE : +81-978-67-3531 FAX : +81-978-67-3532

Feb. 4, 2016

Japan Bio Science Laboratory Co., Ltd
Quality Assurance Section

Certificate

We confirm that the branched cyclodextrin used in "ChalCurb-P8" is identity preserved non-GMO.

-Note-

Ingredient	Agricultural product used	Purpose
Branched Cyclodextrin	Non-GMO corn	Vehicle

2014/11/17

Ensuiko Sugar Refining Co., Ltd.

「ISOELEAT　P」 information

The reply to your question is as follows,

Grade : Food (Food additive)

Composition : Cyclodextrin 80%, Food material 20%

Material : Potato starch & Corn starch

ENSUIKO SUGAR REFINING CO.,LTD.

MATERIAL SAFETY DATA SHEET

Product : ISOELEAT P — No. E-008
Date : March 23, 2007 — Page: 1/5

1. CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

1.1 PRODUCT NAME : ISOELEAT P
1.2 CHEMICAL NATURE : Carbohydrate
1.3 CHEMICAL FAMILY : Cyclodextrin
1.4 MANUFACTURE NAME AND ADDRESS :
Ensuiko Sugar Refining Co., LTD.
2-9-6, Horidome-cho, Nihonbashi Chuo-ku,
Tokyo, 103-0012 Japan
1.5 EMERGENCY TELEPHONE :
+81-45-780-1922
Carbohydrate Research Laboratory
Ensuiko Sugar Refining Co., LTD.

2. COMPOSITION, INFORMATION ON INGREDIENTS

2.1 COMPONENTS : Maltosyl α-Cyclodextrin (G2-α-CD),
Maltosyl β-Cyclodextrin (G2-β-CD),
Dimaltosyl α-Cyclodextrin (G2,G2-α-CD),
Dimaltosyl β-Cyclodextrin (G2,G2-β-CD) :
≧80% as total CDs, ≧50% as G2-CDs
Other components are parent CDs, maltose, dextrin.

2.2 CHEMICAL FORMULA :
G2-α-CD : $C_{48}H_{80}O_{40}$ (MW=1297.12)
G2-β-CD : $C_{54}H_{90}O_{45}$ (MW=1459.27)
G2,G2-α-CD : $C_{60}H_{100}O_{50}$ (MW=1621.41)
G2,G2-β-CD : $C_{66}H_{110}O_{55}$ (MW=1783.55)

2.3 CAS No. : G2-α-CD : 100837-30-9
G2-β-CD : 104723-60-6

3. HAZARDS IDENTIFICATION

No particular hazards for human and environmental

ENSUIKO SUGAR REFINING CO.,LTD.

MATERIAL SAFETY DATA SHEET

Product : ISOELEAT P — No. E-008
Date : March 23, 2007 — Page: 2/5

4. FIRST AID MEASURES

4.1 GENERAL INFORMATION
No particular precaution necessary

4.2 IN CASE OF CONTACT WITH EYES :
Irrigate with clean water, but it's no harmful.

4.3 IN CASE OF CONTACT WITH SKIN :
No particular precaution necessary

4.4 IN CASE OF INHALATION :
Excessive inhalation of dust can impede respiration due to hygroscopic properties.

4.5 IN CASE OF INGESTION :
No particular precaution necessary

5. FIRE FIGHTING MEASURES

5.1 FIRE/EXPLOSION HAZARDS :
N/A(Product will burn when in contact with a flame.)

5.2 EXTINGUISHED MEDIA :
Water, Foam, Dry chemical, CO_2

5.3 SPECIAL FIRE FIGHTING PROCEDURES :
None.

5.4 UNUSUAL FIRE AND EXPLOSION HAZARDS :
In common with most organic materials, this product should be treated as a combustible dust in the finely divided and suspended state.

6. ACCIDENTAL RELEASE MEASURES

6.1 STEPS TO BE TAKEN IN CASE MATERIAL IS RELEASED OR SPILLED :
Sweep or vacuum. Note that an extreme slip hazard can be develop if material spilled on the floor becomes wet.

6.2 WASTE DISPOSAL METHOD :
Handle as none-hazardous material.

ENSUIKO SUGAR REFINING CO.,LTD.

MATERIAL SAFETY DATA SHEET

Product : ISOELEAT P — No. E-008
Date : March 23, 2007 — Page: 3/5

7. HANDLING AND STORAGE

7.1 HANDLING :
No particular precaution necessary.

7.2 STORAGE :
Store in a cool, dry place to maintain best product performance.

8. PRECAUTIONS FOR SAFE USE

8.1 RESPIRATORY PROTECTION :
A dust respirator should be worn if dust becomes annoying.

8.2 VENTILATION :
Local exhaust.
Sufficient to remove airborne dust if handling results in dust generation.

8.3 PROTECTIVE GLOVES :
Not necessary.

8.4 EYE PROTECTION
Goggles recommended heavy dust concentrations.

9. PHYSICAL/CHEMICAL CHARACTERISTICS

9.1 APPEARANCE :
White powder

9.2 SOLUBILITY :
Water-soluble.

9.3 BOILING POINT :
None volatile.

9.4 FLASH POINT :
N/A

9.5 MELTING POINT
255~265 C

9.6 FLAMMABILITY :
N/A
(Product will burn when in contact with a flame, self-extinguishes when ignition source is removed)

9.7 EXPROSIVE CHARACTERISTICS :
N/A

ENSUIKO SUGAR REFINING CO.,LTD.

MATERIAL SAFETY DATA SHEET

Product : ISOELEAT P — No. E-008
Date : March 23, 2007 — Page: 4/5

10. STABILITY AND REACTIVITY

10.1 CONDITIONS TO AVOID :
Storage conditions : As high temperature and a high level of humidity

10.2 MATERIAL TO AVOID :
Strong oxidizing agents.

10.3 HAZARDOUS POLYMERIZATION :
Will not occur.

11. TOXICOLOGICAL INFORMATION

11.1 BACTERIAL MUTATION ASSAY :
G2-α-CD : No mutagenic activity[1]
G2-β-CD : No mutagenic activity[1]

11.2 ACUTE ORAL TOXICITY (Rats)
G2-α-CD : The acute lethal oral dose is greater than 2.0g/kgBW[1]
G2-β-CD : The acute lethal oral dose is greater than 5.0g/kgBW[1]

12. ECOLOGICAL INFORMATION

BIOLOGICAL DECOMPOSITION :
The product is biodegradable.

13. DISPOSAL CONSIDERATIONS

Waste material under observation of natural or regional law.

14. TRANSPORT INFORMATION

The product is a non hazard product.
No special measures have to be observed by transportation.

15. REGULATORY INFORMATION

Mandatory labeling(self-classification) of hazardous preparation : N/A
The regulatory information given in this document only indicates the principle regulations applicable to the product described in the Safety Data Sheet.
The users attention is drawn to the possible existence of additional provisions with completes these regulations.

ENSUIKO SUGAR REFINING CO.,LTD.

MATERIAL SAFETY DATA SHEET

Product : ISOELEAT P — No. E-008
Date : March 23, 2007 — Page: 5/5

16. FURTHER INFORMATION

REFERENCES

1) Huntingdon Research Centre Report

The above mentioned information is based on the actual state of knowledge and does not involve any guaranty of attribute.
Existing laws and regulations have to be observed in own responsibility of the buyer of our product.

SPECIFICATION OF PRODUCT

Ensuiko Sugar Refining Co.,Ltd.
2-9-6 Nihonbashi Horidome-cho,
Chuo-ku, Tokyo, 103-0012 Japan

Product Name : ISOELEAT-P

TEST REQUIRED	SPECIFICATION
Appearance	White powder, odrless, a little sweet and high solubility in water
Purity	
Total-cyclodextrin	NLT 80.0%
Maltosyl-cyclodextrin	NLT 50.0%
Loss on drying	NMT 7.0%
Sulphated ash	NMT 0.3%
pH	4.5~6.5
DE (Dextrose Equivalent)	5~10
Heavy metal (as lead)	NMT 4μg/g
Arsenic	NMT 1μg/g
Viable count	
Bacteria	NMT 300 count/g
Mold and Yeast	NMT 20 count/g
E. coli	Negative

2014/12/11

Ensuiko Sugar Refining Co., Ltd.

Certificate

The reply to your question is as follows,

"ISOELEAT P" is food grade.

Appendix 3. Microscopy and HP-TLC ID Documentation

Certificate Issued To:
JBSL- USA
1547 Palos Verdes Mall #131
Walnut Creek, CA 94597
USA

Work performed at:
Alkemist Labs
1260 Logan Ave B2
Costa Mesa, CA 92626
714-754-HERB (4372)
714-668-9972 (FAX)
sales@alkemist.com
www.alkemist.com

Certificate of Analysis: Angelica keiskei (vendor supplied crude Raw material) (ChalCurbStem020916)

Macroscopy & Microscopy with Digital Photo-Documentation

1

2

3

Company Name:	JBSL- USA
Title:	Angelica keiskei (vendor supplied crude Raw material)
Plant Part:	leaf
Sample Received:	2/12/2016
Sample Description:	Clear Reclosable Plastic Bag
Form of Botanical:	whole/dry
Appearance:	(1) fragments of dark green dried leaf
Lot :	ChalCurbStem020916
Sample :	FAZ04316JBSL4_2
Latin Name:	Angelica keiskei
Reference Sample :	FAZ11612HH1 Angelica keiskei authenticated by macroscopic, microscopic &/or TLC studies according to the reference sources cited below; held at Alkemist Labs, Costa Mesa, CA.
Analyst:	E. Sudberg & N. Popejoy
Magnification:	(2) 400X
Chemical Reagents:	(2) acidified chloral hydrate glycerol solution
Sample Findings:	(2) distinct striations radiating from stomata
Magnification:	(3) 400X
Chemical Reagents:	(3) acidified chloral hydrate glycerol solution
Sample Findings:	(3) jigsaw shaped epidermal cells
Reference Source:	Internal Reference Sample MIC-SOP-54-04, MIC-SOP-54-05, MIC-SOP-54-06, MIC-SOP-510-07

Comments & Conclusions: This sample is representative of Angelica keiskei leaf based on an authenticated reference sample and the consistent characteristic cellular structure of a leaf as well as the reference cited above. The characteristic cellular structures identified in this sample are the distinct striations radiating from stomata seen in micrograph (2) above. In micrograph (3) we see the jigsaw shaped epidermal cells. **This test sample, Angelica keiskei (vendor supplied crude Raw material) (ChalCurbStem020916), is consistent with the microscopic characteristics of the reference samples of Angelica keiskei used above & is characteristic of Angelica keiskei leaf.**

NOTE: The presence of soluble excipients and other plant species material was not detected in this test sample.

Elan Sudberg
Chief Executive Officer

Digitally signed by Elan Sudberg
DN: cn=Elan Sudberg, o=Alkemists Laboratories, ou, email=Elan@alkemist.com, c=US
Date: 2016.02.29 14:15:42 -08'00'

Analyzed by: Nicholas Popejoy
Examined, Reviewed & Authorized by: Elan M Sudberg, CEO & Microscopist, Alkemist Labs

Report Date: 2/25/2016

ISO/IEC 17025
ACCREDITED
CERTIFICATE #3651.01

Certificate Issued To:
JBSL- USA
1547 Palos Verdes Mall #131
Walnut Creek, CA 94597
USA

Work performed at:
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Costa Mesa, CA 92626
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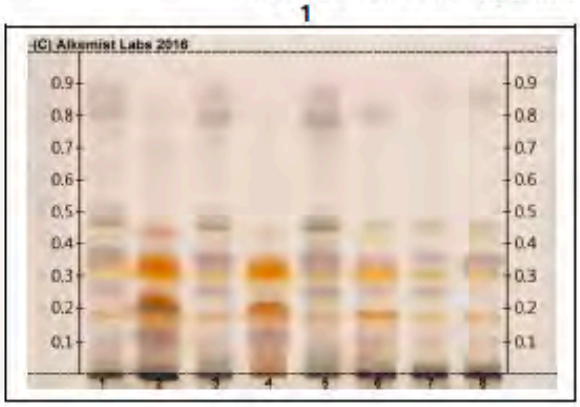
714-668-9972 (FAX)
sales@alkemist.com
www.alkemist.com

Certificate of Analysis: Angelica keiskei (vendor supplied crude Raw material) (ChalCurbStem020916)

High Performance Thin-Layer Chromatography with Photo-Documentation

1



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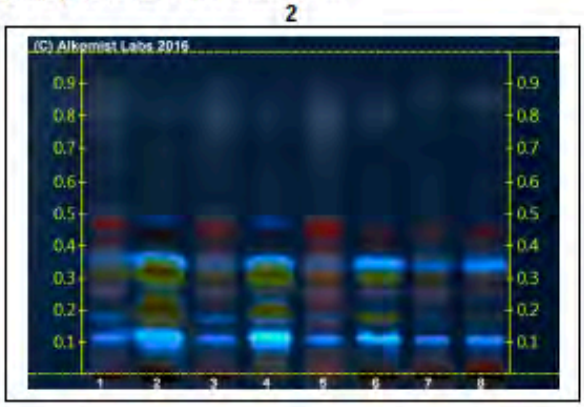


Company Name:	JBSL- USA
Title:	Angelica keiskei (vendor supplied crude Raw material)
Plant Part:	entire
Sample Received:	02/12/16
Sample Description:	Clear Reclosable Plastic Bag
Form of Botanical:	fresh plant specimen
Appearance:	green fresh plant specimen
Source Location:	Japan Bio Science Laboratory Co., Ltd./na
Lot :	(ChalCurbStem020916) → Lane 1(5µl)
Sample :	FAZ04316JBSL1_1
Latin Name:	Angelica keiskei
Reference Sample :	Lane 3(5µl) (FAZ04316JBSL1) Angelica keiskei (entire); Lane 4(5µl) (FAZ04316JBSL3) Angelica keiskei (resin); Lane 5(5µl) (FAZ04316JBSL4) Angelica keiskei (leaf); Lane 6(5µl) (FAZ04316JBSL5) Angelica keiskei (stem); Lane 7(5µl) (FAZ12311PRM1) Angelica keiskei (aerial part); Lane 8(5µl) (FAZ11612HH1) Angelica keiskei (herb (leaf, stem)); held at Alkemist Labs, Costa Mesa, CA.
Analyst:	N. Hoang, L. Scott, P. Fast, T. Collins 66300
Sample Prep:	0.3g+ 3mL CH_3OH sonicate/heat @~50° C ~ 1/2 hr.
Stationary Phase:	Silica gel 60, F_{254}, HPTLC plates
Mobile Phase:	toluene: ethyl acetate: HCOOH [9/1/0.1]
Detection:	(1) 10% Ethanolic H_2SO_4 → 120° C 10 min → visible light (2) 10% Ethanolic H_2SO_4 → 120° C 10 min → UV 365 nm
Reference Source:	Method Developed by Alkemist Labs IDT-SOP-72-01

Comments & Conclusions: Lane 1 is the test sample Angelica keiskei (vendor supplied crude Raw material) (ChalCurbStem020916). Lanes 3, 4, 5, 6, 7, 8 are the reference samples used for comparison. This test sample, Angelica keiskei (vendor supplied crude Raw material) (ChalCurbStem020916), is consistent with the chromatographic profile of the reference samples of Angelica keiskei, used above. **This test sample, Angelica keiskei (vendor supplied crude Raw material) (ChalCurbStem020916) has characteristics of Angelica keiskei entire.**

NOTE: The above conclusion may be a function of the natural variance found in botanicals &/or the extraction process used to create specific extracts. The growing and drying conditions, age, seasonal variations, geographic location, extraction solvents, etc. all play a role in the phytochemical fingerprint of botanicals as well as their extracts; hence, chromatographic variations are expected.

Examined, Reviewed & Authorized by: Sandy Sudberg, Senior Data Analyst, Alkemist Labs Report Date: 02/26/16

ISO/IEC 17025
ACCREDITED
CERTIFICATE #3851.01

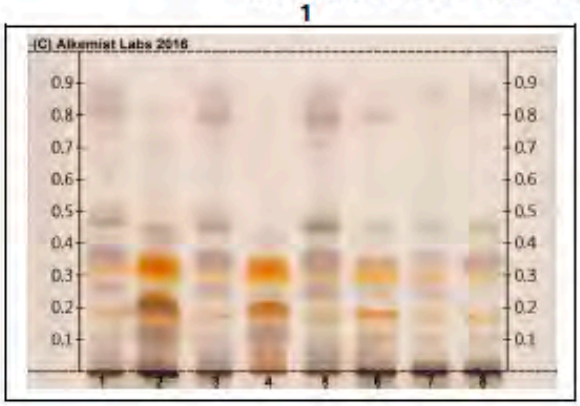
Certificate Issued To:
JBSL- USA
1547 Palos Verdes Mall #131
Walnut Creek, CA 94597
USA

Work performed at:
Alkemist Labs
1260 Logan Ave B2
Costa Mesa, CA 92626
714-754-HERB (4372)
714-668-9972 (FAX)
sales@alkemist.com
www.alkemist.com

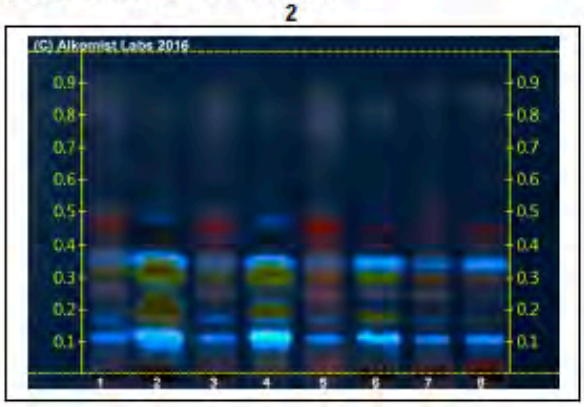
Certificate of Analysis: CHALCURB - P8 (IACA5601)

High Performance Thin-Layer Chromatography with Photo-Documentation

1



2



Company Name:	JBSL- USA
Title:	CHALCURB - P8
Plant Part:	resin
Sample Received:	02/12/16
Sample Description:	Foil Pouch
Form of Botanical:	powdered extract
Appearance:	Foil Pouch
Source Location:	Japan Bio Science Laboratory Co., Ltd.
Lot :	(IACA5601) → Lane 2(5µl)
Sample :	FAZ04316JBSL2_1
Latin Name:	Angelica keiskei
Reference Sample :	Lane 3(5µl) (FAZ04316JBSL1) Angelica keiskei (entire); Lane 4(5µl) (FAZ04316JBSL3) (ChalCurbStem020916) (Vendor Supplied Reference Material) Angelica keiskei (resin); Lane 5(5µl) (FAZ04316JBSL4) Angelica keiskei (leaf); Lane 6(5µl) (FAZ04316JBSL5) Angelica keiskei (stem); Lane 7(5µl) (FAZ12311PRM1) Angelica keiskei (aerial part); Lane 8(5µl) (FAZ11612HH1) Angelica keiskei (herb (leaf, stem)); held at Alkemist Labs, Costa Mesa, CA.
Analyst:	N. Hoang, L. Scott, P. Fast, T. Collins 66300
Sample Prep:	0.3g+ 3ml CH_3OH sonicate/heat @~50° C ~ 1/2 hr.
Stationary Phase:	Silica gel 60, F_{254}, HPTLC plates
Mobile Phase:	toluene: ethyl acetate: HCOOH [9/1/0.1]
Detection:	(1) 10% Ethanolic H_2SO_4 → 120° C 10 min → visible light (2) 10% Ethanolic H_2SO_4 → 120° C 10 min → UV 365 nm
Reference Source:	Method Developed by Alkemist Labs IDT-SOP-72-01

Comments & Conclusions: Lane 2 is the test sample CHALCURB - P8 (IACA5601). Lanes 3, 4, 5, 6, 7, 8 are the reference samples used for comparison. This test sample, CHALCURB - P8 (IACA5601), is compared to and consistent with the chromatographic profile of the vendor supplied reference samples of Angelica keiskei (ChalCurbStem020916), used above. **This test sample, CHALCURB - P8 (IACA5601) has characteristics of the vendor supplied reference material Angelica keiskei (ChalCurbStem020916) resin.**

NOTE: The above conclusion may be a function of the natural variance found in botanicals &/or the extraction process used to create specific extracts. The growing and drying conditions, age, seasonal variations, geographic location, extraction solvents, etc. all play a role in the phytochemical fingerprint of botanicals as well as their extracts; hence, chromatographic variations are expected.

Examined, Reviewed & Authorized by: Sandy Sudberg, Senior Data Analyst, Alkemist Labs Report Date: 02/26/16

ISO/IEC 17025
ACCREDITED
CERTIFICATE #3851.01

Note: Any unidentified lanes in the above chromatograms are confidential and may represent internal studies or other test samples not related to IACA5601.

Appendix 4. Method of Analysis

Japan Bio Science Laboratory Co., Ltd.
URL : http://www.jbsl-net.com E-mail : kaihatu@jbsl-net.com
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1-4-40 Fukushima, Fukushima-ku, Osaka-city, Osaka 553-0003 JAPAN
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PHONE: +81-978-67-3531 FAX : +81-978-67-3532
Japan Bio Science Laboratory

E−211
1st ed.

ASSAY METHOD OF CHALCONE − HPLC Method −	
Reference Code : JBSL-QC	Reference No. : E−211
Date issued : Jul. 21, 2009	Edition : The first edition

REAGENTS

(1) 4-Hydroxyderricin standard [Note 1]
(2) Xanthoangelol standard [Note 1]
(3) Methanol (for HPLC grade)
(4) Distilled water (for HPLC grade)
(5) Ethyl acetate

APPARATUS

(1) High Performance Liquid Chromatograph (HPLC) with UV detector
(2) Test tube (with screw) (φ16 × 150 mm)
(3) 300 mL separatory funnel
(4) 200 mL kjeldahl flask , short neck
(5) Shaker AW-1 (AS ONE)
(6) Rotary evaporator
(7) Membrane filter DISMIC-13HP (ADVANTEC) pore size 0.45 μm

PROCEDURE

(1) Preparation of 4-Hydroxyderricin Standard Solution
Dissolve 10 mg of 4-Hydroxyderricin standard into 10 mL of 80 % methanol. Dilute by adding 80 % methanol and adjust to 0.1 mg/mL, 0.2 mg/mL, 0.3 mg/mL, 0.4 mg/mL and 0.5 mg/mL respectively.

Japan Bio Science Laboratory Co., Ltd.
URL : http://www.jbsl-net.com E-mail : kaihatu@jbsl-net.com
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1-4-40 Fukushima, Fukushima-ku, Osaka-city, Osaka 553-0003 JAPAN
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■FACTORY & LABORATORY
2022 Shioya, Akimachi, Kunisaki-city, Oita 873-0212 JAPAN
PHONE: +81-978-67-3531 FAX : +81-978-67-3532

(2) Preparation of Xanthoangelol Standard Solution

Dissolve 10 mg of Xanthoangelol standard into 10 mL of 80 % methanol. Dilute by adding 80 % methanol and adjust to 0.1 mg/mL, 0.2 mg/mL, 0.5 mg/mL, 0.8 mg/mL and 1.0 mg/mL respectively.

(3) Making Standard Curve

Inject 10 μL of 4-Hydroxyderricin standard solution adjusted to 0.1 mg/mL, 0.2 mg/mL, 0.3 mg/mL, 0.4 mg/mL, 0.5 mg/mL, and 10 μL of Xanthoangelol standard solution adjusted to 0.1 mg/mL, 0.2 mg/mL, 0.5 mg/mL, 0.8 mg/mL and 1.0 mg/mL into HPLC and measure the peak area value. Execute the measurement 2 times continuously and adopt the average value of obtained two peak area values. Make the standard curve of 4-Hydroxyderricin and Xanthoangelol (E1 and E2) from the peak area value and corresponding solution concentration.

(4) Analytical condition of HPLC

Following is the analytical condition of HPLC.

Column size	:	4.6 mm I.D. × 250 mm length ODS column, STR-ODS II (SHINWA Chemical Industrials, Ltd)
Column temperature	:	50 ℃
Mobile phase	:	Methanol : Distilled water (8 : 2 v/v)
Flow rate	:	0.9 mL/min
Detector	:	UV detector
Detection	:	330 nm
Injection volume	:	10 μL
Analysis time	:	42 minutes

(5) Extraction of Chalcone

【Solids or Powder sample】

Weigh 1.0 g of sample into 100 mL beaker, add 10 mL of distilled water to disperse the sample. Pour the sample solution into separatory funnel, add 100 mL of ethyl acetate and shake with shaker for 10 min at 250 rpm. Stay until the water layer

Japan Bio Science Laboratory Co., Ltd.
URL http://www.jbsl-net.com E-mail kaihatu@jbsl-net.com
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PHONE: +81-6-6451-1711 FAX : +81-6-6451-1712
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PHONE: +81-3-5209-1601 FAX : +81-3-5209-1602
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2022 Shioya, Akimachi, Kunisaki-city, Oita 873-0212 JAPAN
PHONE: +81-978-67-3531 FAX : +81-978-67-3532
Japan Bio Science Laboratory

and the solvent layer is separated. Collected water layer to 100 mL beaker, and transfer the solvent layer into kjeldahl flask, short neck. Return water layer that collected in 100 mL beaker into separatory funnel while the residue is washed with 100 mL of ethyl acetate. Repeat two more times with the same procedure as above, shaking, allowed to stand, the solvent layer fractionation. The solvent layer in kjeldahl flask, short neck is evaporate the solvent with rotary evaporator under reduced pressure. 〔Note Ⅱ〕
Add appropriate volume of ethyl acetate to the concentrate to be dissolved. Filtrate the sample solution with membrane filter.

【Liquid sample】
Weigh 1.0 g of sample into Test tube (with screw), then add 10 mL of ethyl acetate. Mix well and sonicate for 20 minutes. After sonication, mix with a touch mixer for 3 minutes. Stay until the water layer and the solvent layer is separated. Transfer the solvent layer into 50mL volumetric flask with Pasteur pipette.
Add 10 mL of ethyl acetate to the water layer remaining in the test tube. Repeat two more times with the same procedure as above, sonication, allowed to stand, the solvent layer fractionation.
Add ethyl acetate to make 50 mL (M) to solvent layer fraction into 50 mL volumetric flask. Filtrate the sample solution with membrane filter.

(6) DILUTION
【High-concentrated chalcone sample】
Dilute (D) by ethyl acetate, 4-Hydroxyderricin concentration in the sample solution adjust to 0.002 mg/mL to 0.8 mg/mL and Xanthoangelol concentration in the sample solution adjust to 0.002 mg/mL to 0.8 mg/mL.
And use it for HPLC analysis.

【Low-concentrated chalcone sample】
In the case of a powdery or solid sample, the sample solution used in the HPLC analysis without dilute.

Japan Bio Science Laboratory Co., Ltd.
URL : http://www.jbsl-net.com E-mail : kaihatu@jbsl-net.com
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E−21L
1st ed.

However, adjust the amount of ethyl acetate (M) added to dissolve the concentrate, 4-Hydroxyderricin concentration in the sample solution adjust to 0.002 mg/mL to 0.8 mg/mL and Xanthoangelol concentration in the sample solution adjust to 0.002 mg/mL to 0.8 mg/mL.

In the case of liquid sample, after separating the water layer and solvent layer in the extraction operation, collected the solvent layer to kjeldahl flask, short neck with a Pasteur pipette, and the solvent is evaporated under reduced pressure on a rotary evaporator. [Note Ⅱ]

Dilute the condensate in ethyl acetate of appropriate quantity (M) to be 0.002 mg/mL to 0.8 mg/mL by 4-Hydroxyderricin concentration and to be 0.002 mg/mL to 1.8 mg/mL by Xanthoangelol concentration.

Filtrate the lysate with membrane filter.

And use it for HPLC analysis as the sample solution.

(7) Measurement

Peak area of the sample is measured by HPLC.

Execute the measurement 2 times continuously and adopt the average value of obtained two peak area values of the Xanthoangelol and 4-Hydroxyderricin (P1 and P2).

Dilute the sample solution accordingly in case chalcone concentration of the sample solution is high.

The detection limit of 4-Hydroxyderricin and Xanthoangelol is 0.002 mg/mL together.

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E-211
1st ed.

CALCULATION

Using the standard curve was created with Xanthoangelol standard and 4-Hydroxyderricin standard, calculated by the following equation, Xanthoangelol concentration, and 4-Hydroxyderricin concentration in the sample solution.

(1) 4-Hydroxyderricin concentration (mg/g) $= C_1 \times M \times D \times 1/W$

(2) Xanthoangelol concentration (mg/g) $= C_2 \times M \times D \times 1/W$

(3) Total Chalcone concentration (mg/g)

= 4-Hydroxyderricin concentration + Xanthoangelol concentration

C_1 : Concentration of 4-Hydroxyderricin (mg/mL)
Substituted peak area value (P1) to the standard curve (E1) and calculate it.

C_2 : Concentration of Xanthoangelol (mg/mL)
Substituted peak area value (P2) to the standard curve (E2) and calculate it.

M : Amount of solvent needed to dissolve (mL)

D : Dilution rate 〔Note Ⅲ〕

W : Sample weight (g)

Notes

Ⅰ. Use the purified standard, Purity is more than 99.0 %.

Ⅱ. In case moisture remains, add benzene : ethanol (8 : 2 v/v) and evaporate the water to completely distil away moisture.

Ⅲ. Because of the low concentration of the chalcone sample, in the case of used in HPLC analysis without dilution the sample solution, " Dilution rate " is " 1 ".

Appendix 5. Representative Certificates of Analysis

Appendix 5.1	Certificate of Analysis for JBSL-USA's Ashitaba Chalcone Powder Lot IACA6120
Appendix 5.2	Certificate of Analysis for JBSL-USA's Ashitaba Chalcone Powder Lot IACA6628
Appendix 5.3	Certificate of Analysis for JBSL-USA's Ashitaba Chalcone Powder Lot IACA7105
Appendix 5.4	Certificate of Analysis for JBSL-USA's Ashitaba Chalcone Powder Lot IACA8521
Appendix 5.5	Certificate of Analysis for JBSL-USA's Ashitaba Chalcone Powder Lot IACA9910

Appendix 5.1 Certificate of Analysis for JBSL-USA's Ashitaba Chalcone Powder Lot IACA6120

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PHONE : +81-978-67-3531 FAX : +81-978-67-3532

ChalCurb

Certificate of Analysis

Product Name : ChalCurb-P8
Lot No. : IACA6120
Exp. Date : Jan. 19, 2019
Manuf. Date : Jan. 20, 2016
Batch Size : 408.5 kg
Description : Powder of Ashitaba (*Angelica keiskei*) sap.

Matters	Specification	Result	Test Method
Identity (*)	Detection of Xanthoangelol and 4-Hydroxyderricin.	Conforms	HPLC method
Total Chalcones	NLT 8.0 %	8.19 %	HPLC method
Loss on Drying	NMT 8.0 %	2.1 %	1 g, 105℃, 4 hour
Total Viable Aerobic Count	NMT 1,000 CFU/g	NMT 300 CFU/g	U.S.FDA BAM (Chapter 3)
Coliforms	NMT 30 CFU/g	NMT 10 CFU/g	ISO 4382-1991
Yeast & Mold	NMT 100 CFU/g	NMT 10 CFU/g	U.S.FDA BAM (Chapter 18)
Salmonella	Negative /25g	Negative	AOAC sec.967.26
Escherichia coli	Negative /g	Negative	U.S.FDA BAM (Chapter 4a)
Lead	NMT 1 ppm	NMT 0.01 ppm	ICP/MS
Arsenic	NMT 1 ppm	NMT 0.1 ppm	ICP/MS
Mercury	NMT 1 ppm	NMT 0.01 ppm	ICP/MS
Cadmium	NMT 1 ppm	0.003 ppm	ICP/MS

*: We identify Xanthoangelol and 4-Hydroxyderricin.

Mar. 18, 2016

Japan Bio Science Laboratory Co., LTD.

[redacted]

Kei Takizawa / MANAGER
Quality Assurance Section

Appendix 5.2 Certificate of Analysis for JBSL-USA's Ashitaba Chalcone Powder Lot IACA6628

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PHONE : +81-978-67-3531 FAX : +81-978-67-3532

ChalCurb

Certificate of Analysis

Product Name : ChalCurb-P8
Lot No. : IACA6628
Exp. Date : Jun. 27, 2019
Manuf. Date : Jun. 28, 2016
Batch Size : 477 kg
Description : Powder of Ashitaba (*Angelica keiskei*) sap.

Matters	Specification	Result	Test Method
Identity (*)	Detection of Xanthoangelol and 4-Hydroxyderricin.	Conforms	HPLC method
Total Chalcones	NLT 8.0 %	8.03 %	HPLC method
Loss on Drying	NMT 8.0 %	1.9 %	1 g, 105℃, 4 hour
Total Viable Aerobic Count	NMT 1,000 CFU/g	NMT 300 CFU/g	U.S.FDA BAM (Chapter 3)
Coliforms	NMT 30 CFU/g	NMT 10 CFU/g	ISO 4382:1991
Yeast & Mold	NMT 100 CFU/g	NMT 10 CFU/g	U.S.FDA BAM (Chapter 18)
Salmonella	Negative /25g	Negative	AOAC sec.967.26
Escherichia coli	Negative /g	Negative	U.S.FDA BAM (Chapter 4a)
Lead	NMT 1 ppm	0.016 ppm	ICP/MS
Arsenic	NMT 1 ppm	NMT 0.1 ppm	ICP/MS
Mercury	NMT 1 ppm	NMT 0.01 ppm	ICP/MS
Cadmium	NMT 1 ppm	NMT 0.002 ppm	ICP/MS

*: We identify Xanthoangelol and 4-Hydroxyderricin.

Jul. 29, 2016

Japan Bio Science Laboratory Co., LTD.

[redacted]

Kei Takizawa / MANAGER
Quality Assurance Section

Appendix 5.3 Certificate of Analysis for JBSL-USA's Ashitaba Chalcone Powder Lot IACA7105

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PHONE: +81-978-67-3531 FAX : +81-978-67-3532

ChalCurb

Certificate of Analysis

Product Name : ChalCurb-P8
Lot No. : IACA7105
Exp. Date : Jan. 4. 2020
Manuf. Date : Jan. 5, 2017
Batch Size : 503 kg
Description : Powder of Ashitaba (*Angelica keiskei*) sap.

Matters	Specification	Result	Test Method
Identity (*)	Detection of Xanthoangelol and 4-Hydroxyderricin	Conforms	HPLC method
Total Chalcones	NLT 8.0 %	8.7 %	HPLC method
Loss on Drying	NMT 8.0 %	1.4 %	1 g, 105℃, 4 hour
Total Viable Aerobic Count	NMT 1,000 CFU/g	NMT 300 CFU/g	U.S.FDA BAM (Chapter 3)
Coliforms	NMT 30 CFU/g	NMT 10 CFU/g	ISO 4382:1991
Yeast & Mold	NMT 100 CFU/g	NMT 10 CFU/g	U.S.FDA BAM (Chapter 18)
Salmonella	Negative /25g	Negative	AOAC sec.967.26
Escherichia coli	Negative /g	Negative	U.S.FDA BAM (Chapter 4a)
Lead	NMT 1 ppm	0.016 ppm	ICP/MS
Arsenic	NMT 1 ppm	NMT 0.1 ppm	ICP/MS
Mercury	NMT 1 ppm	NMT 0.01 ppm	ICP/MS
Cadmium	NMT 1 ppm	NMT 0.002 ppm	ICP/MS

*: We identify Xanthoangelol and 4-Hydroxyderricin.

Mar. 2, 2017

Japan Bio Science Laboratory Co., LTD.

[redacted]

Kei Takizawa / MANAGER
Quality Assurance Section

Appendix 5.4 Certificate of Analysis for JBSL-USA's Ashitaba Chalcone Powder Lot IACA8521

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PHONE: +81-978-67-3531 FAX : +81-978-67-3532

ChalCurb

Certificate of Analysis

Product Name : ChalCurb-P8
Lot No. : IACA8521
Exp. Date : May. 20, 2021
Manuf. Date : May. 21, 2018
Batch Size : 450.9 kg
Description : Powder of Ashitaba (*Angelica keiskei*) sap.

Matters	Specification	Result	Test Method
Identity (*)	Detection of Xanthoangelol and 4-Hydroxyderricin.	Conforms	HPLC method
Total Chalcones Xanthoangelol 4-Hydroxyderricin	NLT 8.0 %	8.45 % 5.35 % 3.10 %	HPLC method
Loss on Drying	NMT 8.0 %	2.5 %	1 g, 105℃, 4 hour
Total Viable Aerobic Count	NMT 1,000 CFU/g	NMT 30 CFU/g	U.S.FDA BAM (Chapter 3)
Coliforms	NMT 30 CFU/g	NMT 10 CFU/g	ISO 4382:1991
Yeast & Mold	NMT 100 CFU/g	NMT 10 CFU/g	U.S.FDA BAM (Chapter 18)
Salmonella	Negative /25g	Negative	AOAC sec.967.26
Escherichia coli	Negative /g	Negative	U.S.FDA BAM (Chapter 4a)
Lead	NMT 1 ppm	NMT 0.01 ppm	ICP/MS
Arsenic	NMT 1 ppm	NMT 0.1 ppm	ICP/MS
Mercury	NMT 1 ppm	NMT 0.01 ppm	ICP/MS
Cadmium	NMT 1 ppm	NMT 0.004 ppm	ICP/MS

*: We identify Xanthoangelol and 4-Hydroxyderricin.

Jul. 20, 2018

Japan Bio Science Laboratory Co., LTD.

[redacted]

Kei Takizawa / MANAGER
Quality Assurance Section

Appendix 5.5 Certificate of Analysis for JBSL-USA's Ashitaba Chalcone Powder Lot IACA9910

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ChalCurb

Certificate of Analysis

Product Name : ChalCurb-P8
Lot No. : IACA9910
Exp. Date : Sep. 9, 2022
Manuf. Date : Sep. 10, 2019
Batch Size : 378.5 kg
Description : Powder of Ashitaba (*Angelica keiskei*) sap.

Matters	Specification	Result	Test Method
Identity (*)	Detection of Xanthoangelol and 4-Hydroxyderricin.	Conforms	HPLC method
Total Chalcones	NLT 8.0 %	8.19 %	HPLC method
Loss on Drying	NMT 8.0 %	2.9 %	1 g, 105℃, 4 hour
Total Viable Aerobic Count	NMT 1,000 CFU/g	NMT 300 CFU/g	U.S.FDA BAM (Chapter 3)
Coliforms	NMT 30 CFU/g	NMT 10 CFU/g	ISO 4382:1991
Yeast & Mold	NMT 100 CFU/g	NMT 10 CFU/g	U.S.FDA BAM (Chapter 18)
Salmonella	Negative /25g	Negative	AOAC sec.967.26
Escherichia coli	Negative /g	Negative	U.S.FDA BAM (Chapter 4a)
Lead	NMT 1 ppm	NMT 0.05 ppm	ICP/MS
Arsenic	NMT 1 ppm	NMT 0.1 ppm	ICP/MS
Mercury	NMT 1 ppm	NMT 0.005 ppm	ICP/MS
Cadmium	NMT 1 ppm	NMT 0.01 ppm	ICP/MS

*: We identify Xanthoangelol and 4-Hydroxyderricin.

Oct. 25, 2019

Japan Bio Science Laboratory Co., LTD.

[redacted]

Kei Takizawa / MANAGER
Quality Assurance Section

Appendix 6. Representative Pesticides Analysis

Appendix 6.1 Certificate of Analysis for JBSL-USA's *A. keiskei* sap Lot 120326
Appendix 6.2 Certificate of Analysis for JBSL-USA's *A. keiskei* sap Lot 130522
Appendix 6.3 Certificate of Analysis for JBSL-USA's *A. keiskei* sap Lot 150521

Appendix 6.1 Certificate of Analysis for JBSL-USA's *A. keiskei* sap Lot 120326

MASIS Certificate of Analysis

Date : 2012/04/16

Customer : Japan Bio Science Laboratory Co., LTD.

CERTIFICATE OF ANALYSIS

MASIS Inc.
FOOD & DRUG NanoAnalysis
2-2-7 Ougi-machi, Hirosaki, Aomori
036-8104 Japan
Phone +81-172-29-1777
Fax +81-172-29-1776

Sample Receiving Date : 2012/04/09
Order Number : 53977

Sample Description : *Angelica keiskei* Sap
Lot 120326

THIS IS TO CERTIFY that we did carry out analytical measurements on the provided sample with the above-mentioned details. The measurement result(s) for the requested analytical item(s) are found as per ANALYTICAL RESULTS. This certificate refers to our intervention and findings on the supplied samples at the time and place mentioned above only. Therefore, it should not be regarded as to certify nor represent the population of the relevant samples.

ANALYTICAL RESULTS

Date of Analysis : From: 2012/04/09 To: 2012/04/16
Item : 340 Items
Summary Results : No items detected out of 340 items
Result Details : As per following list

Method of Analysis : In accordance with analytical methods stipulated in Food Sanitation Act, Method of Analysis in Health Science, Japanese Pharmacopoeia, Japanese Industrial Standards (JIS), PAM*, AOAC, WHO, MASIS, etc as applicable.
*Pesticide Analytical Manual by Food and Drug Administration (FDA)

MQL : Method Quantitation Limit
MDL : Method Detection Limit (1/10 to 1/2 of MQL)
MRL : Maximum Residue Limit according to Japanese Regulation
Unit : ppm = mg/kg
Not Detected : Less than MQL
(for MQL's with ※, this term denotes "Less than MDL")

No. 53977 1/9

MASIS Certificate of Analysis

Details of Analytical Results Order Number 53977

(Unit : ppm)

No.	Item	Result	MQL	MRL	Method
1	BHC	ND	0.01	-	G24
2	DDT	ND	0.01	0.5	G24
3	EPN	ND	0.01	-	G24
4	EPTC	ND	0.01	0.1	G24
5	MCPB	ND	0.01	-	L11
6	XMC	ND	0.01	-	G24
7	ACRINATHRIN	ND	0.01	2	G24
8	AZACONAZOLE	ND	0.01	-	G24
9	AZINPHOS-ETHYL	ND	0.01	-	G24
10	AZINPHOS-METHYL	ND	0.01	0.5	G24
11	ACETAMIPRID	ND	0.01	-	L11
12	ACETOCHLOR	ND	0.01	-	G24
13	ACEPHATE	ND	0.01	0.5	L11
14	AZOXYSTROBIN	ND	0.01	50	L11
15	ATRAZINE	ND	0.01	0.02	G24
16	ANILOFOS	ND	0.01	-	L11
17	AMITRAZ	ND	0.01	-	G24
18	AMETRYN	ND	0.01	-	G24
19	ALACHLOR	ND	0.01	0.01	G24
20	ARAMITE	ND	0.01	0.01	L11
21	ALDRIN/DIELDRIN	ND	0.01	0.1	G24
22	ALLETHRIN	ND	0.01	-	G24
23	ISAZOPHOS	ND	0.01	-	G24
24	ISOXATHION	ND	0.01	0.1	G24
25	ISOFENPHOS	ND	0.01	0.02	G24
26	ISOPROCARB	ND	0.01	-	L11
27	ISOPROTHIOLANE	ND	0.01	-	G24
28	INABENFIDE	ND	0.01	-	G24
29	IPRODIONE	ND	0.01	5.0	L11
30	IPROVALICARB	ND	0.01	-	L11
31	IPROBENFOS	ND	0.01	-	G24
32	IMAZAQUIN	ND	0.01	0.05	G24
33	IMAZAMETHABENZ METHYL ESTER	ND	0.01	-	G24
34	IMAZALIL	ND	0.01	0.02	L11
35	IMIBENCONAZOLE	ND	0.01	-	G24
36	INDANOFAN	ND	0.01	-	L11
37	INDOXACARB	ND	0.01	14	L11
38	UNICONAZOLE P	ND	0.01	-	G24
39	ESPROCARB	ND	0.01	-	G24
40	ETHALFLURALIN	ND	0.01	-	G24
41	ETHION	ND	0.01	0.3	G24
42	ETHIPROLE	ND	0.01	-	G24
43	EDIFENPHOS	ND	0.01	-	G24
44	ETOXAZOLE	ND	0.01	-	G24
45	ETHOXYQUIN	ND	0.01	0.05	G24
46	ETOFENPROX	ND	0.01	2	G24
47	ETHOFUMESATE	ND	0.01	0.1	G24
48	ETHOPROPHOS	ND	0.01	-	G24
49	ETOBENZANID	ND	0.01	-	G24
50	ETRIMFOS	ND	0.01	0.2	G24
51	ENDOSULFAN	ND	0.01	0.5	G24

No. 53977 2/9

MASIS Certificate of Analysis

Details of Analytical Results Order Number 53977

(Unit : ppm)

No.	Item	Result	MQL	MRL	Method
52	ENDRIN	ND	0.01	0.01	G24
53	OXADIAZON	ND	0.01	-	G24
54	OXAZICLOMEFONE	ND	0.01	-	L11
55	OXYFLUORFEN	ND	0.01	0.05	G24
56	OXPOCONAZOLE FUMARATE	ND	0.01	-	G24
57	OMETHOATE	ND	0.01	1	L11
58	ORYZALIN	ND	0.01	-	G24
59	CADUSAFOS	ND	0.01	-	G24
60	CAFENSTROLE	ND	0.01	-	G24
61	CAPTAFOL	ND	※ 0.01	< MDL (<0.01)	G24
62	CARBARYL	ND	0.01	10	L11
63	CARFENTRAZONE-ETHYL	ND	0.01	2	G24
64	CARPROPAMID	ND	0.01	-	L11
65	CARBOXIN	ND	0.01	-	G24
66	CARBOSULFAN	ND	0.01	1	G24
67	CARBOFURAN	ND	0.01	0.5	L11
68	QUIZALOFOP-ETHYL	ND	0.01	0.3	L11
69	QUINALPHOS	ND	0.01	0.05	G24
70	QUINOXYFEN	ND	0.01	-	G24
71	QUINOCLAMINE	ND	0.01	0.03	G24
72	CHINOMETHIONAT	ND	0.01	0.3	G24
73	CAPTAN	ND	0.01	5	G24
74	QUINTOZENE	ND	0.01	0.02	G24
75	COUMAPHOS	ND	※ 0.01	< MDL (<0.01)	L11
76	KRESOXIM-METHYL	ND	0.01	30	G24
77	CLOQUINTOCET-MEXYL	ND	0.01	-	L11
78	CHLOZOLINATE	ND	0.01	0.05	G24
79	CLOFENTEZINE	ND	0.01	0.02	L11
80	CLOMAZONE	ND	0.01	0.02	G24
81	CLOMEPROP	ND	0.01	-	L11
82	CHLORIMURON ETHYL	ND	0.01	-	L11
83	CHLORTHAL-DIMETHYL	ND	0.01	5	G24
84	CHLORDANE	ND	0.01	0.02	G24
85	CHLORPYRIFOS	ND	0.01	0.01	G24
86	CHLORPYRIFOS METHYL	ND	0.01	0.03	G24
87	CHLORFENAPYR	ND	0.01	2	G24
88	CHLORFENSON	ND	0.01	0.01	G24
89	CHLORFENVINPHOS	ND	0.01	0.5	G24
90	CHLORBUFAM	ND	0.01	0.05	G24
91	CHLORPROPHAM	ND	0.01	0.1	G24
92	CHLORBENSIDE	ND	0.01	0.01	G24
93	CHLOROXURON	ND	0.01	0.05	L11
94	CHLOROTHALONIL	ND	0.01	0.5	G24
95	CHLORONEB	ND	0.01	-	G24
96	CHLOROBENZILATE	ND	0.01	0.02	G24
97	SALITHION	ND	0.01	-	G24
98	CYAZOFAMID	ND	0.01	-	L11
99	CYANAZINE	ND	0.01	0.05	G24
100	CYANOFENPHOS	ND	0.01	-	G24
101	CYANOPHOS	ND	0.01	0.05	G24

No. 53977 3/9

MASIS Certificate of Analysis

Details of Analytical Results Order Number 53977

(Unit : ppm)

No.	Item	Result	MQL	MRL	Method
102	DIAFENTHIURON	ND	0.01	0.02	G24
103	DIETHOFENCARB	ND	0.01	5.0	G24
104	DIOXATHION	ND	0.01	0.05	G24
105	CYCLOATE	ND	0.01	-	L11
106	DICLOCYMET	ND	0.01	-	G24
107	DICHLOFENTHION	ND	0.01	0.03	G24
108	DICHLOFLUANID	ND	0.01	5	G24
109	DICLOFOP-METHYL	ND	0.01	-	G24
110	DICLOMEZINE	ND	0.01	0.02	G24
111	DICLORAN	ND	0.01	-	G24
112	DICHLORPROP	ND	0.01	0.05	L11
113	DICHLORVOS/NALED	ND	0.01	0.1	G24
114	DICHLORMID	ND	0.01	-	G24
115	DICOFOL	ND	0.01	3	G24
116	DISULFOTON	ND	0.01	0.5	G24
117	DITHIOPYR	ND	0.01	-	G24
118	CYHALOTHRIN	ND	0.01	0.5	G24
119	CYHALOFOP-BUTYL	ND	0.01	-	G24
120	DIPHENAMID	ND	0.01	-	G24
121	DIPHENYL	ND	0.01	-	G24
122	DIFENOCONAZOLE	ND	0.01	-	G24
123	DIFENZOQUAT	ND	0.01	0.05	G24
124	CYFLUTHRIN	ND	0.01	2.0	G24
125	CYFLUFENAMID	ND	0.01	-	L11
126	DIFLUFENICAN	ND	0.01	0.002	G24
127	CYPROCONAZOLE	ND	0.01	-	G24
128	CYPRODINIL	ND	0.01	30	L11
129	CYPERMETHRIN	ND	0.01	0.05	G24
130	SIMAZINE	ND	0.01	-	G24
131	SIMECONAZOLE	ND	0.01	-	L11
132	DIMETHAMETRYN	ND	0.01	-	G24
133	DIMETHIPIN	ND	0.01	0.04	G24
134	DIMETHYLVINPHOS	ND	0.01	-	G24
135	DIMETHENAMID	ND	0.01	-	G24
136	DIMETHOATE	ND	0.01	1	G24
137	DIMETHOMORPH	ND	0.01	-	L11
138	SIMETRYN	ND	0.01	-	G24
139	DIMEPIPERATE	ND	0.01	-	G24
140	SILAFLUOFEN	ND	0.01	-	L11
141	CINMETHYLIN	ND	0.01	-	G24
142	SPIROXAMINE	ND	0.01	-	G24
143	SPIRODICLOFEN	ND	0.01	-	G24
144	SULPROFOS	ND	0.01	-	L11
145	SETHOXYDIM	ND	0.01	10	L11
146	ZOXAMIDE	ND	0.01	-	G24
147	TERBACIL	ND	0.01	-	G24
148	DIAZINON	ND	0.01	0.1	G24
149	DAIMURON	ND	0.01	-	L11
150	TIADINIL	ND	0.01	-	G24
151	THIAZOPYR	ND	0.01	-	G24
152	THIABENDAZOLE	ND	0.01	2	L11

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Details of Analytical Results Order Number 53977

(Unit : ppm)

No.	Item	Result	MQL	MRL	Method
153	THIOBENCARB	ND	0.01	-	G24
154	THIOMETON	ND	0.01	0.10	G24
155	THIDIAZURON	ND	0.01	-	L11
156	THIFENSULFURON-METHYL	ND	0.01	-	L11
157	THIFLUZAMIDE	ND	0.01	-	G24
158	TECNAZENE	ND	0.01	0.05	G24
159	DESMEDIPHAM	ND	0.01	-	L11
160	TETRACHLORVINPHOS	ND	0.01	0.3	G24
161	TETRACONAZOLE	ND	0.01	-	G24
162	TETRADIFON	ND	0.01	1	G24
163	THENYLCHLOR	ND	0.01	-	G24
164	TEBUCONAZOLE	ND	0.01	-	G24
165	TEBUFENOZIDE	ND	0.01	10	L11
166	TEBUFENPYRAD	ND	0.01	-	G24
167	TEFLUTHRIN	ND	0.01	0.5	G24
168	DEMETON-S-METHYL	ND	0.01	0.4	G24
169	DELTAMETHRIN/TRALOMETHRIN	ND	0.01	0.5	G24
170	TERBUTRYN	ND	0.01	-	G24
171	TERBUFOS	ND	0.01	0.005	G24
172	TRIADIMENOL	ND	0.01	0.2	G24
173	TRIADIMEFON	ND	0.01	0.1	G24
174	TRIALLATE	ND	0.01	0.1	G24
175	TRICHLORFON	ND	0.01	0.50	G24
176	TRICYCLAZOLE	ND	0.01	0.02	G24
177	TRIDEMORPH	ND	0.01	0.05	L11
178	TRIBUPHOS	ND	0.01	-	G24
179	TRIFLUMIZOLE	ND	0.01	1.0	G24
180	TRIFLURALIN	ND	0.01	0.05	G24
181	TRIFLOXYSTROBIN	ND	0.01	3.5	G24
182	TOLYFLOXYSULFURON	ND	0.01	-	L11
183	TOLCLOFOS-METHYL	ND	0.01	2.0	G24
184	TOLFENPYRAD	ND	0.01	-	G24
185	NAPROPAMIDE	ND	0.01	-	G24
186	NITROTHAL-ISOPROPYL	ND	0.01	-	G24
187	NOVALURON	ND	0.01	-	L11
188	NORFLURAZON	ND	0.01	-	G24
189	BARBAN	ND	0.01	0.05	G24
190	PACLOBUTRAZOL	ND	0.01	-	G24
191	VAMIDOTHION	ND	0.01	0.05	G24
192	PARATHION	ND	0.01	0.05	G24
193	PARATHION-METHYL	ND	0.01	1.0	G24
194	HALFENPROX	ND	0.01	-	G24
195	BIORESMETHRIN	ND	0.01	0.1	G24
196	PICOLINAFEN	ND	0.01	-	G24
197	BITERTANOL	ND	0.01	0.05	G24
198	BIFENOX	ND	0.01	-	G24
199	BIFENTHRIN	ND	0.01	-	G24
200	PIPERONYL BUTOXIDE	ND	0.01	0.5	G24
201	PIPEROPHOS	ND	0.01	-	G24
202	PYRACLOFOS	ND	0.01	0.05	G24
203	PYRAZOXYFEN	ND	0.01	-	G24

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Details of Analytical Results Order Number 53977

(Unit : ppm)

No.	Item	Result	MQL	MRL	Method
204	PYRAZOPHOS	ND	0.01	0.05	G24
205	PYRAZOLYNATE	ND	0.01	0.02	L11
206	PYRAFLUFEN ETHYL	ND	0.01	-	G24
207	PYRIDAFENTHION	ND	0.01	0.03	G24
208	PYRIDABEN	ND	0.01	2.0	G24
209	PYRIDALYL	ND	0.01	-	G24
210	PYRIFENOX	ND	0.01	-	G24
211	PYRIFTALID	ND	0.01	-	L11
212	PYRIBUTICARB	ND	0.01	-	G24
213	PYRIPROXYFEN	ND	0.01	-	G24
214	PIRIMICARB	ND	0.01	3	L11
215	PYRIMIDIFEN	ND	0.01	0.05	G24
216	PYRIMINOBAC-METHYL	ND	0.01	-	G24
217	PIRIMIPHOS-METHYL	ND	0.01	1.0	G24
218	PYRIMETHANIL	ND	0.01	-	G24
219	PYROQUILON	ND	0.01	-	G24
220	VINCLOZOLIN	ND	0.01	-	G24
221	FIPRONIL	ND	0.01	0.1	G24
222	FENAMIPHOS	ND	0.01	0.1	G24
223	FENARIMOL	ND	0.01	0.5	G24
224	FENITROTHION	ND	0.01	0.2	G24
225	FENOXANIL	ND	0.01	-	G24
226	FENOXAPROP-ETHYL	ND	0.01	0.1	L11
227	FENOXYCARB	ND	0.01	0.05	L11
228	FENOTHIOCARB	ND	0.01	-	G24
229	PHENOTHRIN	ND	0.01	0.02	G24
230	FENOBUCARB	ND	0.01	0.3	L11
231	FERIMZONE	ND	0.01	-	L11
232	FENAMIDONE	ND	0.01	-	G24
233	FENCHLORPHOS	ND	0.01	0.01	G24
234	FENSULFOTHION	ND	0.01	-	G24
235	FENTHION	ND	0.01	-	G24
236	PHENTHOATE	ND	0.01	0.1	G24
237	FENTRAZAMIDE	ND	0.01	-	G24
238	FENVALERATE	ND	0.01	0.50	G24
239	FENPYROXIMATE	ND	0.01	0.5	L11
240	FENBUCONAZOLE	ND	0.01	-	G24
241	FENPROPATHRIN	ND	0.01	-	G24
242	FENPROPIMORPH	ND	0.01	0.05	G24
243	FENHEXAMID	ND	0.01	30	L11
244	FTHALIDE	ND	0.01	-	G24
245	BUTACHLOR	ND	0.01	-	G24
246	BUTAFENACIL	ND	0.01	-	L11
247	BUTAMIFOS	ND	0.01	0.05	G24
248	BUTYLATE	ND	0.01	-	G24
249	BUPIRIMATE	ND	0.01	-	G24
250	BUPROFEZIN	ND	0.01	-	G24
251	FURATHIOCARB	ND	0.01	0.3	L11
252	FLAMPROP-METHYL	ND	0.01	-	G24
253	FURAMETPYR	ND	0.01	0.1	L11
254	FURILAZOLE	ND	0.01	-	G24

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MASIS Certificate of Analysis

Details of Analytical Results Order Number 53977

(Unit : ppm)

No.	Item	Result	MQL	MRL	Method
255	FLUACRYPYRIM	ND	0.01	-	G24
256	FLUAZINAM	ND	0.01	0.1	G24
257	FLUAZIFOP	ND	0.01	0.5	G24
258	FLUQUINCONAZOLE	ND	0.01	-	G24
259	FLUDIOXONIL	ND	0.01	20	G24
260	FLUCYTHRINATE	ND	0.01	0.50	G24
261	FLUSILAZOLE	ND	0.01	-	G24
262	FLUTHIACET-METHYL	ND	0.01	-	G24
263	FLUTOLANIL	ND	0.01	-	G24
264	FLUTRIAFOL	ND	0.01	-	G24
265	FLUVALINATE	ND	0.01	-	G24
266	FLUFENOXURON	ND	0.01	-	L11
267	FLUMIOXAZIN	ND	0.01	-	G24
268	FLUMICLORAC PENTYL	ND	0.01	-	G24
269	FLURIDONE	ND	0.01	0.1	L11
270	PRETILACHLOR	ND	0.01	-	G24
271	PROCHLORAZ	ND	0.01	5	G24
272	PROCYMIDONE	ND	0.01	5	G24
273	PROTHIOFOS	ND	0.01	-	G24
274	PROPACHLOR	ND	0.01	-	G24
275	PROPAZINE	ND	0.01	0.1	G24
276	PROPANIL	ND	0.01	0.1	G24
277	PROPAPHOS	ND	0.01	-	G24
278	PROPARGITE	ND	0.01	3	G24
279	PROPICONAZOLE	ND	0.01	0.05	G24
280	PROPYZAMIDE	ND	0.01	0.1	G24
281	PROHYDROJASMON	ND	0.01	-	G24
282	PROFENOFOS	ND	0.01	0.05	G24
283	PROBENAZOLE	ND	0.01	0.1	L11
284	PROPOXUR	ND	0.01	2	G24
285	PROMETRYN	ND	0.01	0.05	G24
286	BROMOBUTIDE	ND	0.01	-	G24
287	BROMOPROPYLATE	ND	0.01	0.5	G24
288	BROMOPHOS	ND	0.01	-	G24
289	BROMOPHOS ETHYL	ND	0.01	0.05	G24
290	HEXACHLOROBENZENE	ND	0.01	0.01	G24
291	HEXACONAZOLE	ND	0.01	0.02	G24
292	HEXAZINONE	ND	0.01	-	G24
293	HEXYTHIAZOX	ND	0.01	0.5	L11
294	BENALAXYL	ND	0.01	0.05	G24
295	BENOXACOR	ND	0.01	-	G24
296	HEPTACHLOR	ND	0.01	0.03	G24
297	PERMETHRIN	ND	0.01	3.0	G24
298	PENCONAZOLE	ND	0.01	0.05	G24
299	PENCYCURON	ND	0.01	-	L11
300	BENSULIDE	ND	0.01	0.1	G24
301	BENZOFENAP	ND	0.01	-	L11
302	BENDIOCARB	ND	0.01	-	L11
303	PENDIMETHALIN	ND	0.01	0.2	G24
304	PENTOXAZONE	ND	0.01	-	L11
305	BENFLURALIN	ND	0.01	-	G24

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MASIS Certificate of Analysis

Details of Analytical Results Order Number 53977

(Unit : ppm)

No.	Item	Result	MQL	MRL	Method
306	BENFURESATE	ND	0.01	-	G24
307	PHOXIM	ND	0.01	0.02	L11
308	PHOSALONE	ND	0.01	0.5	G24
309	BOSCALID	ND	0.01	0.7	G24
310	FOSTHIAZATE	ND	0.01	0.1	G24
311	PHOSPHAMIDON	ND	0.01	0.2	G24
312	PHOSMET	ND	0.01	1	L11
313	FORMOTHION	ND	0.01	0.02	G24
314	PHORATE	ND	0.01	0.3	G24
315	MALATHION	ND	0.01	2.0	G24
316	MYCLOBUTANIL	ND	0.01	1.0	G24
317	MECARBAM	ND	0.01	0.05	G24
318	METHACRIFOS	ND	0.01	0.05	G24
319	METHABENZTHIAZURON	ND	0.01	-	G24
320	METHAMIDOPHOS	ND	0.01	0.5	L11
321	METALAXYL/MEFENOXAM	ND	0.01	1	G24
322	METHIOCARB	ND	0.01	0.05	L11
323	METHIDATHION	ND	0.01	0.1	G24
324	METHOXYCHLOR	ND	0.01	0.01	G24
325	METHOXYFENOZIDE	ND	0.01	30	L11
326	METHOPRENE	ND	0.01	-	G24
327	METOMINOSTROBIN	ND	0.01	-	G24
328	METOLACHLOR	ND	0.01	0.1	G24
329	METRIBUZIN	ND	0.01	-	G24
330	MEPANIPYRIM	ND	0.01	-	L11
331	MEVINPHOS	ND	0.01	0.5	G24
332	MEFENACET	ND	0.01	-	G24
333	MEFENPYR-DIETHYL	ND	0.01	-	G24
334	MEPRONIL	ND	0.01	-	G24
335	MONOCROTOPHOS	ND	0.01	0.05	G24
336	MOLINATE	ND	0.01	0.02	G24
337	LACTOFEN	ND	0.01	-	L11
338	LINURON	ND	0.01	0.2	L11
339	LUFENURON	ND	0.01	-	L11
340	LENACIL	ND	0.01	0.3	G24

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Details of Analytical Results Order Number 53977

Analytical Method
G24 : GC-MS
L11 : HPLC-MS/MS

Approved by : [redacted]

Manabu Soma (President)

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Appendix 6.2 Certificate of Analysis for JBSL-USA's *A. keiskei* sap Lot 130522

CERTIFICATE OF ANALYSIS

2013/07/04

Customer : Japan Bio Science Laboratory Co., LTD.

MASIS Inc.
FOOD & DRUG NanoAnalysis
2-2-7 Ougi-machi Hirosaki, Aomori
036-8104 Japan
Phone +81-172-29-1777
Fax +81-172-29-1776

Sample Receiving Date 2013/06/27
Order Number 63177
Sample Description *Angelica keiskei* Sap
Lot No.130522
Item 350 Items

Result Overview No items detected out of 350 items

Result Details

No.	Item	Result	MQL	MRL1	MRL2	Method
1	1,1-DICHLORO-2,2-Bis(4-ETHYLPHENYL)ETHANE	ND	0.01	-	0.01	G14
2	1,2-DIBROMO-3-CHLOROPROPANE	ND	0.01	-	-	G14
3	BHC	ND	0.01	-	-	G14
4	DDT	ND	0.01	-	0.5	G14
5	EPN	ND	0.01	-	-	G14
6	EPTC	ND	0.01	-	0.1	G14
7	TCMTB	ND	0.01	-	-	G14
8	XMC	ND	0.01	-	-	G14
9	LINDANE	ND	0.01	-	1	G14
10	ACRINATHRIN	ND	0.01	-	2	G14
11	AZACONAZOLE	ND	0.01	-	-	G14
12	AZAMETHIPHOS	ND	0.01	-	-	L11
13	AZINPHOS-ETHYL	ND	0.01	-	-	G14
14	AZINPHOS-METHYL	ND	0.01	-	-	G14
15	ACETAMIPRID	ND	0.01	-	-	L11
16	ACETOCHLOR	ND	0.01	-	-	G14
17	AZOXYSTROBIN	ND	0.01	-	70	L11
18	ATRAZINE	ND	0.01	-	0.02	G14
19	ANILOFOS	ND	0.01	-	-	L11
20	AMETRYN	ND	0.01	-	-	G14
21	ALACHLOR	ND	0.01	-	-	G14
22	ALANYCARB	ND	0.01	-	0.1	L11
23	ALDOXYCARB	ND	0.01	-	-	L11
24	ALDRIN/DIELDRIN	ND	0.01	-	0.1	G14
25	ISAZOPHOS	ND	0.01	-	-	G14
26	ISOURON	ND	0.01	-	0.02	L11
27	ISOCARBOPHOS	ND	0.01	-	-	G14
28	ISOXADIFEN-ETHYL	ND	0.01	-	-	G14
29	ISOXATHION	ND	0.01	-	0.1	G14
30	ISOXAFLUTOLE	ND	0.01	-	-	L11
31	ISOFENPHOS	ND	0.01	-	0.02	G14
32	ISOPROCARB	ND	0.01	-	-	L11
33	ISOPROTHIOLANE	ND	0.01	-	-	G14
34	IPROVALICARB	ND	0.01	-	-	L11

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No.	Item	Result	MQL	MRL1	MRL2	Method
35	IPROBENFOS	ND	0.01	-	-	G14
36	IMICYAFOS	ND	0.01	-	-	L11
37	INDANOFAN	ND	0.01	-	-	G14
38	UNICONAZOLE P	ND	0.01	-	-	G14
39	ESPROCARB	ND	0.01	-	-	G14
40	ETHAMETSULFURON-METHYL	ND	0.01	-	-	L11
41	ETHALFLURALIN	ND	0.01	-	-	G14
42	ETHIOFENCARB	ND	0.01	-	-	G14
43	ETHION	ND	0.01	-	0.3	G14
44	EDIFENPHOS	ND	0.01	-	-	G14
45	ETOXAZOLE	ND	0.01	-	-	G14
46	ETOFENPROX	ND	0.01	-	2	G14
47	ETHOPROPHOS	ND	0.01	-	-	G14
48	ETOBENZANID	ND	0.01	-	-	G14
49	ETRIDIAZOLE	ND	0.01	-	0.1	G14
50	ETRIMFOS	ND	0.01	-	0.2	G14
51	EPOXICONAZOLE	ND	0.01	-	-	L11
52	ENDRIN	ND	0.01	-	0.01	G14
53	OXADIAZON	ND	0.01	-	-	G14
54	OXADIXYL	ND	0.01	-	5	G14
55	OXAZICLOMEFONE	ND	0.01	-	-	L11
56	OXYCARBOXIN	ND	0.01	-	-	L11
57	OXYFLUORFEN	ND	0.01	-	-	G14
58	OMETHOATE	ND	0.01	-	1	L11
59	2-PHENYLPHENOL	ND	0.01	-	-	G14
60	CAFENSTROLE	ND	0.01	-	-	G14
61	CARTAP/THIOCYCLAM/BENSULTAP	ND	0.01	-	3	G14
62	CARBARYL	ND	0.01	-	10	L11
63	CARFENTRAZONE-ETHYL	ND	0.01	-	2	G14
64	CARPROPAMID	ND	0.01	-	-	L11
65	CARBETAMIDE	ND	0.01	-	-	L11
66	CARBOXIN	ND	0.01	-	-	G14
67	CARBOSULFAN	ND	0.01	-	1	G14
68	QUINALPHOS	ND	0.01	-	0.05	G14
69	QUINOXYFEN	ND	0.01	-	-	G14
70	QUINOCLAMINE	ND	0.01	-	0.03	G14
71	CHINOMETHIONAT	ND	0.01	-	0.3	G14
72	QUINTOZENE	ND	0.01	-	0.02	G14
73	COUMAPHOS	ND	※ 0.01	< MDL <0.01	< MDL <0.01	L11
74	CUMYLURON	ND	0.01	-	-	G14
75	KRESOXIM-METHYL	ND	0.01	-	30	G14
76	CLOQUINTOCET-MEXYL	ND	0.01	-	-	G14
77	CLODINAFOP-PROPARGYL	ND	0.01	-	0.02	G14
78	CHLOZOLINATE	ND	0.01	-	0.05	G14
79	CLOFENCET	ND	0.01	-	-	L11
80	CLOFENTEZINE	ND	0.01	-	0.02	L11
81	CLOMAZONE	ND	0.01	-	0.02	G14
82	CLOMEPROP	ND	0.01	-	-	G14
83	CHLORETHOXYPHOS	ND	0.01	-	-	G14
84	CHLORTHAL-DIMETHYL	ND	0.01	-	5	G14

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No.	Item	Result	MQL	MRL1	MRL2	Method
85	CHLORPYRIFOS	ND	0.01	–	0.01	G14
86	CHLORPYRIFOS METHYL	ND	0.01	–	0.03	G14
87	CHLORFENSON	ND	0.01	–	–	G14
88	CHLORBUFAM	ND	0.01	–	0.05	G14
89	CHLORPROPHAM	ND	0.01	–	0.1	G14
90	CHLORBENSIDE	ND	0.01	–	0.01	G14
91	CHLOROXURON	ND	0.01	–	0.05	L11
92	CHLOROTHALONIL	ND	0.01	–	0.5	G14
93	CHLOROTOLURON	ND	0.01	–	–	G14
94	CHLORONEB	ND	0.01	–	–	G14
95	CHLOROBENZILATE	ND	0.01	–	0.02	G14
96	SALITHION	ND	0.01	–	–	G14
97	CYANOFENPHOS	ND	0.01	–	–	G14
98	CYANOPHOS	ND	0.01	–	0.05	G14
99	DIURON	ND	0.01	–	0.05	L11
100	DIETHOFENCARB	ND	0.01	–	5.0	G14
101	CYENOPYRAFEN	ND	0.01	–	–	L11
102	DIOXATHION	ND	0.01	–	0.05	G14
103	CYCLOATE	ND	0.01	–	–	L11
104	CYCLOXYDIM	ND	0.01	–	0.05	G14
105	DICLOCYMET	ND	0.01	–	–	G14
106	DICROTOPHOS	ND	0.01	–	–	G14
107	DICHLOFENTHION	ND	0.01	–	0.03	G14
108	DICLOBUTRAZOL	ND	0.01	–	–	G14
109	DICHLOFLUANID	ND	0.01	–	5	G14
110	DICHLOBENIL	ND	0.01	–	–	G14
111	DICLOFOP-METHYL	ND	0.01	–	–	G14
112	DICLORAN	ND	0.01	–	–	G14
113	DICHLORVOS/NALED	ND	0.01	–	0.1	G14
114	DICHLORMID	ND	0.01	–	–	G14
115	DICOFOL	ND	0.01	–	3	G14
116	DISULFOTON	ND	0.01	–	0.5	G14
117	DITHIOPYR	ND	0.01	–	–	G14
118	DINICONAZOLE	ND	0.01	–	–	G14
119	CINIDON-ETHYL	ND	0.01	–	–	G14
120	CINOSULFURON	ND	0.01	–	–	L11
121	CYHALOTHRIN	ND	0.01	–	0.5	G14
122	CYHALOFOP-BUTYL	ND	0.01	–	–	G14
123	DIPHENAMID	ND	0.01	–	–	G14
124	DIFENOCONAZOLE	ND	0.01	–	–	G14
125	CYFLUTHRIN	ND	0.01	–	2.0	G14
126	CYFLUFENAMID	ND	0.01	–	–	L11
127	DIFLUFENICAN	ND	0.01	–	0.002	G14
128	DIFLUBENZURON	ND	0.01	–	1	L11
129	CYPROCONAZOLE	ND	0.01	–	–	G14
130	CYPRODINIL	ND	0.01	–	30	L11
131	CYPERMETHRIN	ND	0.01	–	0.05	G14
132	SIMAZINE	ND	0.01	–	–	G14
133	SIMECONAZOLE	ND	0.01	–	–	L11
134	DIMETHAMETRYN	ND	0.01	–	–	G14
135	DIMETHIRIMOL	ND	0.01	–	0.2	G14

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No.	Item	Result	MQL	MRL1	MRL2	Method
136	DIMETHYLVINPHOS	ND	0.01	-	-	G14
137	DIMETHENAMID	ND	0.01	-	-	G14
138	DIMETHOATE	ND	0.01	-	1	G14
139	DIMETHOMORPH	ND	0.01	-	-	L11
140	SIMETRYN	ND	0.01	-	-	G14
141	DIMEPIPERATE	ND	0.01	-	-	G14
142	CYROMAZINE	ND	0.01	-	7.0	L11
143	CINMETHYLIN	ND	0.01	-	-	G14
144	SPIRODICLOFEN	ND	0.01	-	-	G14
145	SULPROFOS	ND	0.01	-	-	G14
146	SULFOSULFURON	ND	0.01	-	-	L11
147	SULFOTEP	ND	0.01	-	-	G14
148	ZOXAMIDE	ND	0.01	-	-	G14
149	TERBACIL	ND	0.01	-	-	G14
150	DIAZINON	ND	0.01	-	0.1	G14
151	DAIMURON	ND	0.01	-	-	L11
152	THIACLOPRID	ND	0.01	-	-	L11
153	THIAZOPYR	ND	0.01	-	-	G14
154	THIABENDAZOLE	ND	0.01	-	2	L11
155	THIAMETHOXAM	ND	0.01	-	3	L11
156	THIOBENCARB	ND	0.01	-	-	G14
157	THIOMETON	ND	0.01	-	0.10	G14
158	THIFLUZAMIDE	ND	0.01	-	-	G14
159	TECNAZENE	ND	0.01	-	0.05	G14
160	DESMEDIPHAM	ND	0.01	-	-	G14
161	TETRACHLORVINPHOS	ND	0.01	-	0.3	G14
162	TETRACONAZOLE	ND	0.01	-	-	G14
163	TETRADIFON	ND	0.01	-	1	G14
164	THENYLCHLOR	ND	0.01	-	-	G14
165	TEBUCONAZOLE	ND	0.01	-	-	G14
166	TEBUTHIURON	ND	0.01	-	0.02	L11
167	TEBUPIRIMFOS	ND	0.01	-	-	L11
168	TEBUFENPYRAD	ND	0.01	-	-	G14
169	TEFLUTHRIN	ND	0.01	-	0.5	G14
170	DEMETON-S-METHYL	ND	0.01	-	0.4	G14
171	DELTAMETHRIN/TRALOMETHRIN	ND	0.01	-	0.5	G14
172	TERBUTRYN	ND	0.01	-	-	G14
173	TERBUFOS	ND	0.01	-	0.005	G14
174	TRALKOXYDIM	ND	0.01	-	-	L11
175	TRIADIMEFON	ND	0.01	-	0.1	G14
176	TRIAZOPHOS	ND	0.01	-	-	G14
177	TRIALLATE	ND	0.01	-	0.1	G14
178	TRICHLAMIDE	ND	0.01	-	0.2	G14
179	TRICHLORFON	ND	0.01	-	0.50	G14
180	TRITICONAZOLE	ND	0.01	-	-	L11
181	TRIBUPHOS	ND	0.01	-	-	G14
182	TRIFLUMURON	ND	0.01	-	0.02	L11
183	TRIFLURALIN	ND	0.01	-	0.05	G14
184	TRIFLOXYSTROBIN	ND	0.01	-	4	G14
185	TOLYFLOXYSULFURON	ND	0.01	-	-	L11
186	TOLYLFLUANID	ND	0.01	-	-	G14

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No.	Item	Result	MQL	MRL1	MRL2	Method
187	TOLCLOFOS-METHYL	ND	0.01	-	2.0	G14
188	TOLFENPYRAD	ND	0.01	-	-	G14
189	NAPROANILIDE	ND	0.01	-	-	L11
190	NAPROPAMIDE	ND	0.01	-	-	G14
191	NICOTINE	ND	0.01	-	-	G14
192	NITRAPYRIN	ND	0.01	-	-	G14
193	NITROTHAL-ISOPROPYL	ND	0.01	-	-	G14
194	NUARIMOL	ND	0.01	-	-	G14
195	NOVALURON	ND	0.01	-	-	L11
196	NORFLURAZON	ND	0.01	-	-	G14
197	PACLOBUTRAZOL	ND	0.01	-	-	G14
198	PARATHION	ND	0.01	-	0.05	G14
199	PARATHION-METHYL	ND	0.01	-	1.0	G14
200	HALFENPROX	ND	0.01	-	-	G14
201	BIORESMETHRIN	ND	0.01	-	0.1	G14
202	PICOLINAFEN	ND	0.01	-	-	G14
203	PINOXADEN	ND	0.01	-	-	G14
204	BIFENOX	ND	0.01	-	-	G14
205	BIFENTHRIN	ND	0.01	-	-	G14
206	PIPERONYL BUTOXIDE	ND	0.01	-	0.5	G14
207	PIPEROPHOS	ND	0.01	-	-	G14
208	PYRACLONIL	ND	0.01	-	-	L11
209	PYRACLOFOS	ND	0.01	-	0.05	G14
210	PYRAZOPHOS	ND	0.01	-	0.05	G14
211	PYRAZOLYNATE	ND	0.01	-	0.02	L11
212	PYRAFLUFEN ETHYL	ND	0.01	-	-	G14
213	PYRIDAFENTHION	ND	0.01	-	0.03	G14
214	PYRIDABEN	ND	0.01	-	2.0	G14
215	PYRIDALYL	ND	0.01	-	-	G14
216	PYRIFENOX	ND	0.01	-	-	G14
217	PYRIFTALID	ND	0.01	-	-	L11
218	PYRIBUTICARB	ND	0.01	-	-	G14
219	PYRIPROXYFEN	ND	0.01	-	-	G14
220	PIRIMICARB	ND	0.01	-	3	G14
221	PYRIMIDIFEN	ND	0.01	-	0.05	G14
222	PYRIMINOBAC-METHYL	ND	0.01	-	-	G14
223	PIRIMIPHOS-METHYL	ND	0.01	-	1.0	G14
224	PYRIMETHANIL	ND	0.01	-	-	G14
225	PYROQUILON	ND	0.01	-	-	G14
226	VINCLOZOLIN	ND	0.01	-	-	G14
227	FAMPHUR	ND	0.01	-	-	G14
228	FIPRONIL	ND	0.01	-	0.1	G14
229	FENAMIPHOS	ND	0.01	-	0.1	G14
230	FENARIMOL	ND	0.01	-	0.5	G14
231	FENITROTHION	ND	0.01	-	0.2	G14
232	FENOXANIL	ND	0.01	-	-	G14
233	FENOXYCARB	ND	0.01	-	0.05	G14
234	FENOTHIOCARB	ND	0.01	-	-	G14
235	PHENOTHRIN	ND	0.01	-	0.02	G14
236	FENOBUCARB	ND	0.01	-	0.3	L11
237	FENAMIDONE	ND	0.01	-	-	G14

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No.	Item	Result	MQL	MRL1	MRL2	Method
238	FENCHLORPHOS	ND	0.01	-	0.01	G14
239	FENSULFOTHION	ND	0.01	-	-	G14
240	FENTHION	ND	0.01	-	-	G14
241	PHENTHOATE	ND	0.01	-	0.1	G14
242	FENTRAZAMIDE	ND	0.01	-	-	G14
243	FENVALERATE	ND	0.01	-	0.50	G14
244	FENBUCONAZOLE	ND	0.01	-	-	G14
245	FENPROPATHRIN	ND	0.01	-	-	G14
246	FENPROPIMORPH	ND	0.01	-	0.05	G14
247	FENHEXAMID	ND	0.01	-	30	L11
248	FTHALIDE	ND	0.01	-	-	G14
249	BUTACHLOR	ND	0.01	-	-	G14
250	BUTAFENACIL	ND	0.01	-	-	G14
251	BUTAMIFOS	ND	0.01	-	0.05	G14
252	BUTYLATE	ND	0.01	-	-	G14
253	BUTROXYDIM	ND	0.01	-	-	G14
254	BUPIRIMATE	ND	0.01	-	-	G14
255	BUPROFEZIN	ND	0.01	-	-	G14
256	FUBERIDAZOLE	ND	0.01	-	-	L11
257	FURATHIOCARB	ND	0.01	-	0.3	G14
258	FLAMPROP-METHYL	ND	0.01	-	-	G14
259	FURAMETPYR	ND	0.01	-	0.1	G14
260	FURILAZOLE	ND	0.01	-	-	G14
261	FLUACRYPYRIM	ND	0.01	-	-	G14
262	FLUOPICOLIDE	ND	0.01	-	25	L11
263	FLUOMETURON	ND	0.01	-	0.02	L11
264	FLUQUINCONAZOLE	ND	0.01	-	-	G14
265	FLUDIOXONIL	ND	0.01	-	20	G14
266	FLUCYTHRINATE	ND	0.01	-	0.50	G14
267	FLUSILAZOLE	ND	0.01	-	-	G14
268	FLUTHIACET-METHYL	ND	0.01	-	-	G14
269	FLUTOLANIL	ND	0.01	-	-	G14
270	FLUTRIAFOL	ND	0.01	-	-	G14
271	FLUVALINATE	ND	0.01	-	-	G14
272	FLUFENACET	ND	0.01	-	-	L11
273	FLUFENOXURON	ND	0.01	-	10	L11
274	FLUMIOXAZIN	ND	0.01	-	-	G14
275	FLUMICLORAC PENTYL	ND	0.01	-	-	G14
276	FLUMETSULAM	ND	0.01	-	-	L11
277	FLURIDONE	ND	0.01	-	0.1	G14
278	PRETILACHLOR	ND	0.01	-	-	G14
279	PROCHLORAZ	ND	0.01	-	5	G14
280	PROCYMIDONE	ND	0.01	-	5	G14
281	PROSULFOCARB	ND	0.01	-	0.1	G14
282	PROTHIOFOS	ND	0.01	-	-	G14
283	PROPAQUIZAFOP	ND	0.01	-	-	L11
284	PROPACHLOR	ND	0.01	-	-	G14
285	PROPAZINE	ND	0.01	-	0.1	G14
286	PROPANIL	ND	0.01	-	0.1	G14
287	PROPAPHOS	ND	0.01	-	-	G14
288	PROPICONAZOLE	ND	0.01	-	0.05	G14

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No.	Item	Result	MQL	MRL1	MRL2	Method
289	PROPYZAMIDE	ND	0.01	–	0.1	G14
290	PROHYDROJASMON	ND	0.01	–	–	G14
291	PROPHAM	ND	※ 0.01	< MDL <0.01	< MDL <0.01	G14
292	PROFENOFOS	ND	0.01	–	0.05	G14
293	PROPETAMPHOS	ND	0.01	–	–	G14
294	PROPOXYCARBAZONE	ND	0.01	–	–	L11
295	PROPOXUR	ND	0.01	–	2	G14
296	BROMACIL	ND	0.01	–	–	G14
297	PROMECARB	ND	0.01	–	–	G14
298	PROMETRYN	ND	0.01	–	0.05	G14
299	BROMOBUTIDE	ND	0.01	–	–	G14
300	BROMOPROPYLATE	ND	0.01	–	0.5	G14
301	BROMOPHOS	ND	0.01	–	–	G14
302	BROMOPHOS ETHYL	ND	0.01	–	0.05	G14
303	HEXACHLOROBENZENE	ND	0.01	–	0.01	G14
304	HEXACONAZOLE	ND	0.01	–	0.02	G14
305	HEXAZINONE	ND	0.01	–	–	G14
306	HEXYTHIAZOX	ND	0.01	–	0.5	L11
307	BENALAXYL	ND	0.01	–	0.05	G14
308	BENOXACOR	ND	0.01	–	–	G14
309	PERMETHRIN	ND	0.01	–	3.0	G14
310	PENCONAZOLE	ND	0.01	–	0.05	G14
311	PENCYCURON	ND	0.01	–	–	L11
312	BENSULFURON METHYL	ND	0.01	–	–	L11
313	BENDIOCARB	ND	0.01	–	–	G14
314	PENTACHLOROPHENOL	ND	0.01	–	–	G14
315	BENTHIAVALICARB ISOPROPYL	ND	0.01	–	–	L11
316	PENDIMETHALIN	ND	0.01	–	0.2	G14
317	PENTOXAZONE	ND	0.01	–	–	G14
318	BENFLURALIN	ND	0.01	–	–	G14
319	BENFURESATE	ND	0.01	–	–	G14
320	PHOSALONE	ND	0.01	–	0.5	G14
321	BOSCALID	ND	0.01	–	0.7	G14
322	PHOSMET	ND	0.01	–	1	G14
323	FONOFOS	ND	0.01	–	–	G14
324	FORAMSULFURON	ND	0.01	–	–	L11
325	FORCHLORFENURON	ND	0.01	–	–	L11
326	FOLPET	ND	0.01	–	–	G14
327	FORMOTHION	ND	0.01	–	0.02	G14
328	PHORATE	ND	0.01	–	0.3	G14
329	MALATHION	ND	0.01	–	2.0	G14
330	MANDIPROPAMID	ND	0.01	–	–	L11
331	MYCLOBUTANIL	ND	0.01	–	1	G14
332	MECARBAM	ND	0.01	–	0.05	G14
333	METHACRIFOS	ND	0.01	–	0.05	G14
334	METHABENZTHIAZURON	ND	0.01	–	–	G14
335	METALAXYL/MEFENOXAM	ND	0.01	–	1	G14
336	METHIDATHION	ND	0.01	–	0.1	G14
337	METHOXYCHLOR	ND	0.01	–	0.01	G14
338	METOSULAM	ND	0.01	–	–	L11

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CERTIFICATE OF ANALYSIS Order Number 63177 2013/07/04

No.	Item	Result	MQL	MRL1	MRL2	Method
339	METSULFURON-METHYL	ND	0.01	-	-	L11
340	METOMINOSTROBIN	ND	0.01	-	-	G14
341	METOLACHLOR	ND	0.01	-	0.1	G14
342	MEVINPHOS	ND	0.01	-	0.5	G14
343	MEFENPYR-DIETHYL	ND	0.01	-	-	G14
344	MEPRONIL	ND	0.01	-	-	G14
345	MONOCROTOPHOS	ND	0.01	-	0.05	G14
346	MONOLINURON	ND	0.01	-	0.05	G14
347	LACTOFEN	ND	0.01	-	-	G14
348	RIMSULFURON	ND	0.01	-	-	L11
349	RESMETHRIN	ND	0.01	-	0.1	G14
350	LENACIL	ND	0.01	-	0.3	G14

Unit : ppm = mg/kg
Result : Result of analysis
ND : Less than Method Quantitation Limit. Note, however, in case of substances stipulated to be "not detected" in foods, this term denotes that the result is less than Method Detection Limit (1/10 to 1/2 of MQL).
MQL : Method Quantitation Limit
※ : Inclusion forbidden in any food: figures represent Method Detection Limit (1/10 to 1/2 of MQL).
MRL : Maximum Residue Limit according to Japanese Regulation
MRL1 : -
MRL2 : MRL for Other umbelliferous vegetables (as of 2013/07/04)
" — " : MRL not specified

Analytical Method
G14 : GC-MS/MS
L11 : HPLC-MS/MS

Approved by: [redacted]
Manabu Soma (President)

Appendix 6.3 Certificate of Analysis for JBSL-USA's *A. keiskei* sap Lot 150521

CERTIFICATE OF ANALYSIS

2015/07/06

Customer : Japan Bio Science Laboratory Co., LTD.

MASIS Inc.
FOOD & DRUG NanoAnalysis
2-2-7 Ougi-machi, Hirosaki, Aomori
036-8104 Japan
Phone +81-172-29-1777
Fax. +81-172-29-1776
ISO/IEC 17025:2005 Accredited Laboratory

Sample Receiving Date 2015/06/29
Order Number 77976
Sample Description *Angelica keiskei* Sap
Lot No.150521
Item 350 Items

Result Overview No items detected out of 350 items

Result Details

No.	Item	Result	MQL	MRL1	MRL2	Method
1	1,1-DICHLORO-2,2-Bis(4-ETHYLPHENYL)ETHANE	ND	0.01	-	-	G14
2	1,2-DIBROMO-3-CHLOROPROPANE	ND	0.01	-	-	G14
3	BHC	ND	0.01	-	-	G14
4	DDT	ND	0.01	-	0.5	G14
5	EPN	ND	0.01	-	-	G14
6	EPTC	ND	0.01	-	0.1	G14
7	TCMTB	ND	0.01	-	-	G14
8	XMC	ND	0.01	-	-	G14
9	γ-BHC	ND	0.01	-	1	G14
10	ACRINATHRIN	ND	0.01	-	2	G14
11	AZACONAZOLE	ND	0.01	-	-	G14
12	AZAMETHIPHOS	ND	0.01	-	-	L11
13	AZINPHOS-ETHYL	ND	0.01	-	-	G14
14	AZINPHOS-METHYL	ND	0.01	-	-	G14
15	ACETAMIPRID	ND	0.01	-	10	L11
16	ACETOCHLOR	ND	0.01	-	-	G14
17	AZOXYSTROBIN	ND	0.01	-	70	L11
18	ATRAZINE	ND	0.01	-	0.02	G14
19	ANILOFOS	ND	0.01	-	-	L11
20	AMETRYN	ND	0.01	-	-	G14
21	ALACHLOR	ND	0.01	-	-	G14
22	ALANYCARB	ND	0.01	-	0.1	L11
23	ALDOXYCARB	ND	0.01	-	-	L11
24	ALDRIN/DIELDRIN	ND	0.01	-	0.1	G14
25	ISAZOPHOS	ND	0.01	-	-	G14
26	ISOURON	ND	0.01	-	0.02	L11
27	ISOCARBOPHOS	ND	0.01	-	-	G14
28	ISOXADIFEN-ETHYL	ND	0.01	-	-	G14

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No.	Item	Result	MQL	MRL1	MRL2	Method
29	ISOXATHION	ND	0.01	-	0.1	G14
30	ISOXAFLUTOLE	ND	0.01	-	-	L11
31	ISOFENPHOS	ND	0.01	-	0.02	G14
32	ISOPROCARB	ND	0.01	-	-	L11
33	ISOPROTHIOLANE	ND	0.01	-	-	G14
34	IPROVALICARB	ND	0.01	-	-	L11
35	IPROBENFOS	ND	0.01	-	-	G14
36	MICYAFOS	ND	0.01	-	-	L11
37	INDANOFAN	ND	0.01	-	-	G14
38	UNICONAZOLE P	ND	0.01	-	-	G14
39	ESPROCARB	ND	0.01	-	-	G14
40	ETHAMETSULFURON-METHYL	ND	0.01	-	-	L11
41	ETHALFLURALIN	ND	0.01	-	-	G14
42	ETHIOFENCARB	ND	0.01	-	-	G14
43	ETHION	ND	0.01	-	0.3	G14
44	EDIFENPHOS	ND	0.01	-	-	G14
45	ETOXAZOLE	ND	0.01	-	-	G14
46	ETOFENPROX	ND	0.01	-	2	G14
47	ETHOPROPHOS	ND	0.01	-	-	G14
48	ETOBENZANID	ND	0.01	-	-	G14
49	ETRIDIAZOLE	ND	0.01	-	0.1	G14
50	ETRIMFOS	ND	0.01	-	-	G14
51	EPOXICONAZOLE	ND	0.01	-	-	L11
52	ENDRIN	ND	0.01	-	0.01	G14
53	OXADIAZON	ND	0.01	-	-	G14
54	OXADIXYL	ND	0.01	-	5	G14
55	OXAZICLOMEFONE	ND	0.01	-	-	L11
56	OXYCARBOXIN	ND	0.01	-	-	L11
57	OXYFLUORFEN	ND	0.01	-	-	G14
58	OMETHOATE	ND	0.01	-	1	L11
59	2-PHENYLPHENOL	ND	0.01	-	-	G14
60	CAFENSTROLE	ND	0.01	-	-	G14
61	CARTAP/THIOCYCLAM/BENSULTAP	ND	0.01	-	3	G14
62	CARBARYL	ND	0.01	-	10	L11
63	CARFENTRAZONE-ETHYL	ND	0.01	-	2	G14
64	CARPROPAMID	ND	0.01	-	-	L11
65	CARBETAMIDE	ND	0.01	-	-	L11
66	CARBOXIN	ND	0.01	-	-	G14
67	CARBOSULFAN	ND	0.01	-	1	G14
68	QUINALPHOS	ND	0.01	-	0.05	G14
69	QUINOXYFEN	ND	0.01	-	-	G14
70	QUINOCLAMINE	ND	0.01	-	0.03	G14
71	CHINOMETHIONAT	ND	0.01	-	0.3	G14
72	QUINTOZENE	ND	0.01	-	0.02	G14
73	COUMAPHOS	ND	※ 0.01	< MDL <0.01	< MDL <0.01	L11
74	CUMYLURON	ND	0.01	-	-	L11

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No. 77976 MASIS 2/9

CERTIFICATE OF ANALYSIS Order Number 77976 2015/07/06

No.	Item	Result	MQL	MRL1	MRL2	Method
75	KRESOXIM-METHYL	ND	0.01	-	-	G14
76	CLOQUINTOCET-MEXYL	ND	0.01	-	-	G14
77	CLODINAFOP-PROPARGYL	ND	0.01	-	0.02	G14
78	CHLOZOLINATE	ND	0.01	-	-	G14
79	CLOFENCET	ND	0.01	-	-	L11
80	CLOFENTEZINE	ND	0.01	-	0.02	L11
81	CLOMAZONE	ND	0.01	-	0.02	G14
82	CLOMEPROP	ND	0.01	-	-	G14
83	CHLORETHOXYPHOS	ND	0.01	-	-	G14
84	CHLORTHAL-DIMETHYL	ND	0.01	-	5	G14
85	CHLORPYRIFOS	ND	0.01	-	0.01	G14
86	CHLORPYRIFOS METHYL	ND	0.01	-	0.03	G14
87	CHLORFENSON	ND	0.01	-	-	G14
88	CHLORBUFAM	ND	0.01	-	-	G14
89	CHLORPROPHAM	ND	0.01	-	0.1	G14
90	CHLORBENSIDE	ND	0.01	-	-	G14
91	CHLOROXURON	ND	0.01	-	-	L11
92	CHLOROTHALONIL	ND	0.01	-	0.5	G14
93	CHLOROTOLURON	ND	0.01	-	-	G14
94	CHLORONEB	ND	0.01	-	-	G14
95	CHLOROBENZILATE	ND	0.01	-	0.02	G14
96	SALITHION	ND	0.01	-	-	G14
97	CYANOFENPHOS	ND	0.01	-	-	G14
98	CYANOPHOS	ND	0.01	-	0.05	G14
99	DIURON	ND	0.01	-	0.05	L11
100	DIETHOFENCARB	ND	0.01	-	5.0	G14
101	CYENOPYRAFEN	ND	0.01	-	-	L11
102	DIOXATHION	ND	0.01	-	-	G14
103	CYCLOATE	ND	0.01	-	-	L11
104	CYCLOXYDIM	ND	0.01	-	0.05	G14
105	DICLOCYMET	ND	0.01	-	-	G14
106	DICROTOPHOS	ND	0.01	-	-	G14
107	DICHLOFENTHION	ND	0.01	-	-	G14
108	DICLOBUTRAZOL	ND	0.01	-	-	G14
109	DICHLOFLUANID	ND	0.01	-	5	G14
110	DICHLOBENIL	ND	0.01	-	-	G14
111	DICLOFOP-METHYL	ND	0.01	-	-	G14
112	DICLORAN	ND	0.01	-	-	G14
113	DICHLORVOS/NALED	ND	0.01	-	0.1	G14
114	DICHLORMID	ND	0.01	-	-	G14
115	DICOFOL	ND	0.01	-	3	G14
116	DISULFOTON	ND	0.01	-	0.5	G14
117	DITHIOPYR	ND	0.01	-	-	G14
118	DINICONAZOLE	ND	0.01	-	-	G14
119	CINIDON-ETHYL	ND	0.01	-	-	G14
120	CINOSULFURON	ND	0.01	-	-	L11

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CERTIFICATE OF ANALYSIS Order Number 77976 2015/07/06

No.	Item	Result	MQL	MRL1	MRL2	Method
121	CYHALOTHRIN	ND	0.01	-	0.5	G14
122	CYHALOFOP-BUTYL	ND	0.01	-	-	G14
123	DIPHENAMID	ND	0.01	-	-	G14
124	DIFENOCONAZOLE	ND	0.01	-	0.5	G14
125	CYFLUTHRIN	ND	0.01	-	2.0	G14
126	CYFLUFENAMID	ND	0.01	-	-	L11
127	DIFLUFENICAN	ND	0.01	-	-	G14
128	DIFLUBENZURON	ND	0.01	-	1	L11
129	CYPROCONAZOLE	ND	0.01	-	-	G14
130	CYPRODINIL	ND	0.01	-	30	L11
131	CYPERMETHRIN	ND	0.01	-	0.05	G14
132	SIMAZINE	ND	0.01	-	-	G14
133	SIMECONAZOLE	ND	0.01	-	-	L11
134	DIMETHAMETRYN	ND	0.01	-	-	G14
135	DIMETHIRIMOL	ND	0.01	-	-	L11
136	DIMETHYLVINPHOS	ND	0.01	-	-	G14
137	DIMETHENAMID	ND	0.01	-	-	G14
138	DIMETHOATE	ND	0.01	-	1	G14
139	DIMETHOMORPH	ND	0.01	-	-	L11
140	SIMETRYN	ND	0.01	-	-	G14
141	DIMEPIPERATE	ND	0.01	-	-	G14
142	CYROMAZINE	ND	0.01	-	7.0	L11
143	CINMETHYLIN	ND	0.01	-	-	G14
144	SPIRODICLOFEN	ND	0.01	-	-	G14
145	SULPROFOS	ND	0.01	-	-	L11
146	SULFOSULFURON	ND	0.01	-	-	L11
147	SULFOTEP	ND	0.01	-	-	G14
148	ZOXAMIDE	ND	0.01	-	-	G14
149	TERBACIL	ND	0.01	-	-	G14
150	DIAZINON	ND	0.01	-	0.1	G14
151	DAIMURON	ND	0.01	-	-	L11
152	THIACLOPRID	ND	0.01	-	-	L11
153	THIAZOPYR	ND	0.01	-	-	G14
154	THIABENDAZOLE	ND	0.01	-	2	L11
155	THIAMETHOXAM	ND	0.01	-	3	L11
156	THIOBENCARB	ND	0.01	-	-	G14
157	THIOMETON	ND	0.01	-	0.10	G14
158	THIFLUZAMIDE	ND	0.01	-	-	G14
159	TECNAZENE	ND	0.01	-	0.05	G14
160	DESMEDIPHAM	ND	0.01	-	-	G14
161	TETRACHLORVINPHOS	ND	0.01	-	0.3	G14
162	TETRACONAZOLE	ND	0.01	-	-	G14
163	TETRADIFON	ND	0.01	-	1	G14
164	THENYLCHLOR	ND	0.01	-	-	G14
165	TEBUCONAZOLE	ND	0.01	-	-	G14
166	TEBUTHIURON	ND	0.01	-	0.02	L11

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No.	Item	Result	MQL	MRL1	MRL2	Method
167	TEBUPIRIMFOS	ND	0.01	-	-	L11
168	TEBUFENPYRAD	ND	0.01	-	-	G14
169	TEFLUTHRIN	ND	0.01	-	0.5	G14
170	DEMETON-S-METHYL	ND	0.01	-	0.4	G14
171	DELTAMETHRIN/TRALOMETHRIN	ND	0.01	-	0.5	G14
172	TERBUTRYN	ND	0.01	-	-	G14
173	TERBUFOS	ND	0.01	-	0.005	G14
174	TRALKOXYDIM	ND	0.01	-	-	L11
175	TRIADIMEFON	ND	0.01	-	0.1	G14
176	TRIAZOPHOS	ND	0.01	-	-	G14
177	TRIALLATE	ND	0.01	-	0.1	G14
178	TRICHLAMIDE	ND	0.01	-	-	G14
179	TRICHLORFON	ND	0.01	-	0.50	G14
180	TRITICONAZOLE	ND	0.01	-	-	L11
181	TRIBUPHOS	ND	0.01	-	-	G14
182	TRIFLUMURON	ND	0.01	-	0.02	L11
183	TRIFLURALIN	ND	0.01	-	0.05	G14
184	TRIFLOXYSTROBIN	ND	0.01	-	4	G14
185	TOLYFLOXYSULFURON	ND	0.01	-	-	L11
186	TOLYLFLUANID	ND	0.01	-	-	G14
187	TOLCLOFOS-METHYL	ND	0.01	-	2.0	G14
188	TOLFENPYRAD	ND	0.01	-	-	G14
189	NAPROANILIDE	ND	0.01	-	-	L11
190	NAPROPAMIDE	ND	0.01	-	-	G14
191	NICOTINE	ND	0.01	-	-	G14
192	NITRAPYRIN	ND	0.01	-	-	G14
193	NITROTHAL-ISOPROPYL	ND	0.01	-	-	G14
194	NUARIMOL	ND	0.01	-	-	G14
195	NOVALURON	ND	0.01	-	-	L11
196	NORFLURAZON	ND	0.01	-	-	G14
197	PACLOBUTRAZOL	ND	0.01	-	-	G14
198	PARATHION	ND	0.01	-	0.05	G14
199	PARATHION-METHYL	ND	0.01	-	1.0	G14
200	HALFENPROX	ND	0.01	-	-	G14
201	BIORESMETHRIN	ND	0.01	-	0.1	G14
202	PICOLINAFEN	ND	0.01	-	-	G14
203	PINOXADEN	ND	0.01	-	-	G14
204	BIFENOX	ND	0.01	-	-	G14
205	BIFENTHRIN	ND	0.01	-	0.05	G14
206	PIPERONYL BUTOXIDE	ND	0.01	-	0.5	G14
207	PIPEROPHOS	ND	0.01	-	-	G14
208	PYRACLONIL	ND	0.01	-	-	L11
209	PYRACLOFOS	ND	0.01	-	0.05	G14
210	PYRAZOPHOS	ND	0.01	-	-	G14
211	PYRAZOLYNATE	ND	0.01	-	0.02	L11
212	PYRAFLUFEN ETHYL	ND	0.01	-	-	G14

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No.	Item	Result	MQL	MRL1	MRL2	Method
213	PYRIDAFENTHION	ND	0.01	-	-	G14
214	PYRIDABEN	ND	0.01	-	-	G14
215	PYRIDALYL	ND	0.01	-	-	G14
216	PYRIFENOX	ND	0.01	-	-	G14
217	PYRIFTALID	ND	0.01	-	-	L11
218	PYRIBUTICARB	ND	0.01	-	-	G14
219	PYRIPROXYFEN	ND	0.01	-	-	G14
220	PIRIMICARB	ND	0.01	-	3	G14
221	PYRIMIDIFEN	ND	0.01	-	-	G14
222	PYRIMINOBAC-METHYL	ND	0.01	-	-	G14
223	PIRIMIPHOS-METHYL	ND	0.01	-	1.0	G14
224	PYRIMETHANIL	ND	0.01	-	-	G14
225	PYROQUILON	ND	0.01	-	-	G14
226	VINCLOZOLIN	ND	0.01	-	-	G14
227	FAMPHUR	ND	0.01	-	-	G14
228	FIPRONIL	ND	0.01	-	0.1	L11
229	FENAMIPHOS	ND	0.01	-	0.1	G14
230	FENARIMOL	ND	0.01	-	0.5	G14
231	FENITROTHION	ND	0.01	-	0.2	G14
232	FENOXANIL	ND	0.01	-	-	G14
233	FENOXYCARB	ND	0.01	-	0.05	G14
234	FENOTHIOCARB	ND	0.01	-	-	G14
235	PHENOTHRIN	ND	0.01	-	0.02	G14
236	FENOBUCARB	ND	0.01	-	0.3	L11
237	FENAMIDONE	ND	0.01	-	-	G14
238	FENCHLORPHOS	ND	0.01	-	-	G14
239	FENSULFOTHION	ND	0.01	-	-	G14
240	FENTHION	ND	0.01	-	-	G14
241	PHENTHOATE	ND	0.01	-	-	G14
242	FENTRAZAMIDE	ND	0.01	-	-	G14
243	FENVALERATE	ND	0.01	-	0.50	G14
244	FENBUCONAZOLE	ND	0.01	-	-	G14
245	FENPROPATHRIN	ND	0.01	-	-	G14
246	FENPROPIMORPH	ND	0.01	-	0.05	G14
247	FENHEXAMID	ND	0.01	-	30	L11
248	FTHALIDE	ND	0.01	-	-	G14
249	BUTACHLOR	ND	0.01	-	-	G14
250	BUTAFENACIL	ND	0.01	-	-	G14
251	BUTAMIFOS	ND	0.01	-	0.05	G14
252	BUTYLATE	ND	0.01	-	-	G14
253	BUTROXYDIM	ND	0.01	-	-	G14
254	BUPIRIMATE	ND	0.01	-	-	G14
255	BUPROFEZIN	ND	0.01	-	-	G14
256	FUBERIDAZOLE	ND	0.01	-	-	L11
257	FURATHIOCARB	ND	0.01	-	0.3	G14
258	FLAMPROP-METHYL	ND	0.01	-	-	G14

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No.	Item	Result	MQL	MRL1	MRL2	Method
259	FURAMETPYR	ND	0.01	-	-	G14
260	FURILAZOLE	ND	0.01	-	-	G14
261	FLUACRYPYRIM	ND	0.01	-	-	G14
262	FLUOPICOLIDE	ND	0.01	-	25	L11
263	FLUOMETURON	ND	0.01	-	0.02	L11
264	FLUQUINCONAZOLE	ND	0.01	-	-	G14
265	FLUDIOXONIL	ND	0.01	-	20	G14
266	FLUCYTHRINATE	ND	0.01	-	0.50	G14
267	FLUSILAZOLE	ND	0.01	-	-	G14
268	FLUTHIACET-METHYL	ND	0.01	-	-	G14
269	FLUTOLANIL	ND	0.01	-	-	G14
270	FLUTRIAFOL	ND	0.01	-	-	G14
271	FLUVALINATE	ND	0.01	-	-	G14
272	FLUFENACET	ND	0.01	-	-	L11
273	FLUFENOXURON	ND	0.01	-	10	L11
274	FLUMIOXAZIN	ND	0.01	-	-	G14
275	FLUMICLORAC PENTYL	ND	0.01	-	-	G14
276	FLUMETSULAM	ND	0.01	-	-	L11
277	FLURIDONE	ND	0.01	-	-	G14
278	PRETILACHLOR	ND	0.01	-	-	G14
279	PROCHLORAZ	ND	0.01	-	5	G14
280	PROCYMIDONE	ND	0.01	-	5	G14
281	PROSULFOCARB	ND	0.01	-	0.1	G14
282	PROTHIOFOS	ND	0.01	-	-	G14
283	PROPAQUIZAFOP	ND	0.01	-	-	L11
284	PROPACHLOR	ND	0.01	-	-	G14
285	PROPAZINE	ND	0.01	-	0.1	G14
286	PROPANIL	ND	0.01	-	0.1	G14
287	PROPAPHOS	ND	0.01	-	-	G14
288	PROPICONAZOLE	ND	0.01	-	5	G14
289	PROPYZAMIDE	ND	0.01	-	0.1	G14
290	PROHYDROJASMON	ND	0.01	-	-	G14
291	PROPHAM	ND	※ 0.01	< MDL <0.01	< MDL <0.01	G14
292	PROFENOFOS	ND	0.01	-	0.05	G14
293	PROPETAMPHOS	ND	0.01	-	-	G14
294	PROPOXYCARBAZONE	ND	0.01	-	-	L11
295	PROPOXUR	ND	0.01	-	2	G14
296	BROMACIL	ND	0.01	-	-	G14
297	PROMECARB	ND	0.01	-	-	G14
298	PROMETRYN	ND	0.01	-	0.05	G14
299	BROMOBUTIDE	ND	0.01	-	-	G14
300	BROMOPROPYLATE	ND	0.01	-	0.5	G14
301	BROMOPHOS	ND	0.01	-	-	G14
302	BROMOPHOS ETHYL	ND	0.01	-	-	G14
303	HEXACHLOROBENZENE	ND	0.01	-	0.01	G14
304	HEXACONAZOLE	ND	0.01	-	0.02	G14

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No.	Item	Result	MQL	MRL1	MRL2	Method
305	HEXAZINONE	ND	0.01	-	-	G14
306	HEXYTHIAZOX	ND	0.01	-	0.5	L11
307	BENALAXYL	ND	0.01	-	0.05	G14
308	BENOXACOR	ND	0.01	-	-	G14
309	PERMETHRIN	ND	0.01	-	3.0	G14
310	PENCONAZOLE	ND	0.01	-	0.05	G14
311	PENCYCURON	ND	0.01	-	-	L11
312	BENSULFURON METHYL	ND	0.01	-	-	L11
313	BENDIOCARB	ND	0.01	-	-	G14
314	PENTACHLOROPHENOL	ND	0.01	-	-	G14
315	BENTHIAVALICARB ISOPROPYL	ND	0.01	-	-	L11
316	PENDIMETHALIN	ND	0.01	-	0.2	G14
317	PENTOXAZONE	ND	0.01	-	-	G14
318	BENFLURALIN	ND	0.01	-	-	G14
319	BENFURESATE	ND	0.01	-	-	G14
320	PHOSALONE	ND	0.01	-	0.5	G14
321	BOSCALID	ND	0.01	-	5	G14
322	PHOSMET	ND	0.01	-	1	G14
323	FONOFOS	ND	0.01	-	-	G14
324	FORAMSULFURON	ND	0.01	-	-	L11
325	FORCHLORFENURON	ND	0.01	-	-	L11
326	FOLPET	ND	0.01	-	-	G14
327	FORMOTHION	ND	0.01	-	-	G14
328	PHORATE	ND	0.01	-	0.3	G14
329	MALATHION	ND	0.01	-	2	G14
330	MANDIPROPAMID	ND	0.01	-	-	L11
331	MYCLOBUTANIL	ND	0.01	-	1	G14
332	MECARBAM	ND	0.01	-	-	G14
333	METHACRIFOS	ND	0.01	-	-	G14
334	METHABENZTHIAZURON	ND	0.01	-	-	G14
335	METALAXYL/MEFENOXAM	ND	0.01	-	1	G14
336	METHIDATHION	ND	0.01	-	0.1	G14
337	METHOXYCHLOR	ND	0.01	-	0.01	G14
338	METOSULAM	ND	0.01	-	-	L11
339	METSULFURON-METHYL	ND	0.01	-	-	L11
340	METOMINOSTROBIN	ND	0.01	-	-	G14
341	METOLACHLOR	ND	0.01	-	0.1	G14
342	MEVINPHOS	ND	0.01	-	0.5	G14
343	MEFENPYR-DIETHYL	ND	0.01	-	-	G14
344	MEPRONIL	ND	0.01	-	-	G14
345	MONOCROTOPHOS	ND	0.01	-	0.05	G14
346	MONOLINURON	ND	0.01	-	-	G14
347	LACTOFEN	ND	0.01	-	-	G14
348	RIMSULFURON	ND	0.01	-	-	L11
349	RESMETHRIN	ND	0.01	-	0.1	G14
350	LENACIL	ND	0.01	-	0.3	G14

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CERTIFICATE OF ANALYSIS

Order Number 77976

2015/07/06

Unit	:	ppm = mg/kg
Result	:	Result of analysis
ND	:	Less than Method Quantitation Limit. Note, however, in case of substances stipulated to be "not detected" in foods, this term denotes that the result is less than Method Detection Limit (1/10 to 1/2 of MQL).
MQL	:	Method Quantitation Limit
※	:	Inclusion forbidden in any food; figures represent Method Detection Limit (1/10 to 1/2 of MQL).
MRL	:	Maximum Residue Limit according to Japanese Regulation
MRL1	:	–
MRL2	:	MRL for Other umbelliferous vegetables (as of 2015/06/29)
" — "	:	No specific MRL is specified

Analytical Method

G14 : GC-MS/MS
L11 : HPLC-MS/MS

Approved by :

Manabu Soma (President)

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Appendix 7. NHANES Food Codes

Soft Drinks

92400000	Soft drink, NFS
92410310	Soft drink, cola
92410320	Soft drink, cola, diet
92410340	Soft drink, cola, decaffeinated
92410350	Soft drink, cola, decaffeinated, diet
92410360	Soft drink, pepper type
92410370	Soft drink, pepper type, diet
92410410	Soft drink, cream soda
92410510	Soft drink, fruit flavored, caffeine free
92410520	Soft drink, fruit flavored, diet, caffeine free
92410550	Soft drink, fruit flavored, caffeine containing
92410560	Soft drink, fruit flavored, caffeine containing, diet
92410310	Soft drink, ginger ale
92410710	Soft drink, root beer
92410720	Soft drink, root beer, diet
92411510	Soft drink, cola, fruit or vanilla flavored
92411610	Soft drink, cola, fruit or vanilla flavored, diet

Jelly, Jams, Preserves and Marmalade

91401000	Jelly, all flavors
91402000	Jam, preserve, all flavors
91405000	Jelly, sugar free, all flavors
9140600	Jam, preserve, marmalade, sugar free, all flavors

Candy Containing Chocolate

91703040	Caramel candy, chocolate covered

91703060	Caramel with nuts, chocolate covered
91703070	Rolo
91703200	TWIX Caramel Cookie Bars
91705010	Milk chocolate candy, plain
91705020	Milk chocolate cady, with cereal
91705030	Kit Kat
91705040	Chocolate, milk, with nuts, not almond or peanuts
91705060	Milk chocolate candy, with almonds
91705200	Chocolate, semi-sweet morsel
91707010	Fondant, chocolate covered
91713030	Fudge, chocolate
91715100	SNICKERS Bar
91718100	Butterfinger
91726130	MILKY WAY Bar
91731010	M&M's Peanut Chocolate Candies
91734100	Reese's Peanut Butter Cup
91746100	M&M's Milk Chocolate Candies
91760500	Truffles

Butter

81100500	Butter, NFS
81101000	Butter, stick, salted
81101010	Butter, whipped, tub, salted

Appendix 8. *In vivo* Studies

Numerous *in vivo* studies have been conducted on ashitaba and its constituents. While many studies did not monitor specific safety endpoints or report on adverse effects, the observed metabolism, health effects, and/or biological activity of these studies may reveal potential safety related concerns. A summary of these published studies is provided in the table below.

Appendix 8 Table 1. *In vivo* studies conducted on *A. keiskei*-derived test articles

Investigation	Animal Model	Test Material and Study Duration	Results and Observations	Comments	Reference
Anti-tumor activity	8-week-old female ICR mice with TPA-enhanced DMBA-initiated tumors	1 mg 4-HD or 1 mg XA applied topically, 2X per week for 20 weeks	4-HD and XA suppressed the action of the tumor promoter	No adverse effects reported	Okuyama et al. (1991)
Anti-hyperlipidemic effect	Sprague-Dawley rats	i.p. injection of 100 mg per kg methanol extract of *A. keiskei* aerial parts or 5 mg per kg luteolin-7-O-β-D-glucoside isolated from *A. keiskei* aerial parts daily for 2 weeks	Rats treated with *A. keiskei* methanolic extract and luteolin-7-O-β-D-glucoside had significantly lower total serum cholesterol, LDL cholesterol, atherosclerotic index, total lipid, and triglyceride values compared to the hyperlipidemic control group	Article in Korean	Park et al. (1995)
Anti-hyperlipidemic effect	Male Sprague Dawley rats	Rats were fed diets containing 5% *A. keiskei* flour for 6 weeks.	Rats fed the *A. keiskei* supplemented diet had decreased plasma total cholesterol, LDL, and triglycerides levels. A decrease in triglycerides in the liver and increases in fecal cholesterol, total neural steroid, and bile acid were also observed.	Article in Korean	Park et al. (1997)
Anti-tumor activity	Female BALB/c mice	100 g ashitaba leaf was boiled in 2 L of water for 30 minutes to prepare an extract of 30 mg per mL. Mice were fed a standard diet of RMI or RMI supplemented with 0.93 mL of extract for 14 days prior to injection with 130,000 tumor cells in the flank.	There was no significant difference in food consumption or weight gain between the control and ashitaba leaf extract groups. Consumption of ashitaba leaf extract reduced tumor growth and prevented tumor development in some mice.	There was one unscheduled mouse death in the control group. No adverse effects were reported.	Sigurdsson et al. (2005)
Anti-tumor activity	Mice with TPA-enhanced DMBA-initiated tumors	85 nM isobavachalcone topically, 2X per week for 20 weeks	Isobavachalcone suppressed the action of the papilloma promoter	No adverse effects reported	Akihisa et al. (2006)
Anti-hyperlipidemic effect	Rats	Rats were fed a standard diet supplemented with 5% *A. keiskei*	Treatment with ashitaba powder significantly lowered serum total	Article in Korean	Choe et al. (2007)

Investigation	Animal Model	Test Material and Study Duration	Results and Observations	Comments	Reference
		powder for eight weeks.	cholesterol, LDL, and VLDL levels, while an increase was observed in HDL levels. There was no observed effect on serum triglyceride levels. Both quercetin and isoquercetin were detected in serum and liver, whereas hyperoside was not detected in rats supplemented with ashitaba powder.		
Effect on quality and composition of chicken eggs	560 ISA brown laying hens	Hens were fed dried dietary whole dried *A. keiskei* (0.1% or 0.3%) or 0.3% *A. keiskei* 'peel' for 12 weeks.	There were no differences between the control and supplemented hens with respect to egg production, feed intake, or egg mass. Egg yolk color, but not egg or eggshell quality, was improved with *A. keiskei* supplementation. A non-statistically significant increase in the vitamin and polyunsaturated fatty acid concentration of eggs in the *A. keiskei* treatment groups was also observed.	Article in Korean	Kang et al. (2008)
Anti-hyperlipidemic effect	Female Sprague-Dawley rats	Rats were fed a high cholesterol diet for 8 weeks, followed by basal diet (control) or high cholesterol diet with and without supplementation with turmeric extract, *A. keiskei* extract, or turmeric and *A. keiskei* extracts together.	Total serum cholesterol and LDL-cholesterol decreased in the *A. keiskei* extract and turmeric plus *A. keiskei* groups compared to control.	Article in Korean	Kim et al. (2008)
		To induce hypertriglyceridemia, i.p. injection of 0.5 g per kg bw P-	Serum triglycerides were decreased in the *A. keiskei* extract		

Investigation	Animal Model	Test Material and Study Duration	Results and Observations	Comments	Reference
		407 was administered once every 3 days. Rats were fed a basal diet, high cholesterol diet, turmeric extract, *A. keiskei* extract, or turmeric and *A. keiskei* extracts together.	and turmeric plus *A. keiskei* groups compared to control.		
Anti-hepatotoxic effect	7-week-old male Sprague-Dawley rats	Methanolic ashitaba extract prepared from dried, powdered aerial parts, and was suspended in 0.5 carboxymethylcellulose. Rats were treated with 200 or 500 mg per kg bw ashitaba extract or vehicle control via feeding needle daily for 7 days before i.p. administration of 1 g per kg bw D-galactosamine (GalN) or 1 mL per kg bw carbon tetrachloride (CCl_4) on day 7.	After 24-hours of hepatotoxin administration, rats treated with ashitaba extract had reduced GalN-induced elevation in liver lipid peroxidation, plasma-aspartate-transaminase, and alanine-transaminase activities. Treatment with 500 mg per kg bw ashitaba extract prevented GalN-induced elevation in triglyceride, total cholesterol, and LDL levels. Treatment with ashitaba extract exacerbated the effects of CCl_4, as further elevation in aspartate-transaminase, alanine-transaminase, and lipid peroxidation was observed.	No adverse effects reported	Choi and Park (2011)
Anti-tumor activity	6- to 8-week old Kunming mice	Mice were inoculated with H22 hepatocarcinoma cells and were divided into five treatment groups (n=10 mice per group): saline (control); 5, 20, or 40 mg per kg bw per day chalcone (90% purity) by intragastric lavage; or 20 mg per kg cyclophosphamide via i.p. injection once every other day for 10 days.	Significant reductions of the proliferation rate of hepatocarcinoma cells and expression levels of proliferating cell nuclear antigen and BCL-2 protein were observed in mice in the 20 and 40 mg per kg bw per day chalcone treatment groups compared with control.	No adverse effects reported	He et al. (2011)

Investigation	Animal Model	Test Material and Study Duration	Results and Observations	Comments	Reference
Glucose uptake promotion	7-week-old ICR mice	Single dose, oral administration of 50 mg per kg bw or 250 mg per kg bw ethanol acetate extract of ashitaba chalcone powder containing 150.6 mg per g 4-HD and 146.0 mg per g XA 60 minutes prior to glucose loading (1 g per kg bw)	Blood glucose analyzed at -60, 0, 15, 30, 60, and 120 minutes after glucose administration; Ashitaba extract suppressed acute hyperglycemia	No adverse effects reported	Kawabata et al. (2011)
Anti-thrombotic effect	7-week-old male kwl ICR mice	i.p. injection of: 100 mg per kg ashitaba stem sap; 25 mg per kg bw ashitaba stem sap ethyl acetate extract; 10 mg per kg bw ashitaba stem sap chalcone-rich fraction; 10 mg per kg bw ashitaba stem sap coumarin-rich fraction; 5 mg per kg bw XA; or 5 mg per kg bw 4-HD daily for 7 days, followed by an i.p. injection of 0.05 mg per kg bw lipopolysaccharides to induce plasminogen activator inhibitor-1 (PAI-1) production	Lipopolysaccharide-induced PAI-1 production was inhibited in mice treated with ashitaba stem sap ethyl acetate extract, the chalcone-rich fraction, and XA.	No adverse effects reported	Ohkura et al. (2011)
		Oral administration of ashitaba stem sap suspended in corn oil at 100 mg per mouse for 6 weeks, followed by a subcutaneous injection of 0.01 mg per kg lipopolysaccharides	Lipopolysaccharide-induced PAI-1 production was inhibited in mice treated with ashitaba stem sap		
Atopic dermatitis inhibition	1-Fluoro-2,4-dininitrobenzene (DNFB)-induced C57BL/6 mice	Topical administration of aqueous extract, 50% ethanol extract, 100% ethanol extract, and the fresh juice of *A. keiskei*	All treatments reduced ear thickness levels and ear epidermis against swelling by DNFB inducement. Inflammatory cytokine IL-4 was inhibited in the juice-treated group, and IL-13 was	Article in Korean	Kim et al. (2012b)

Investigation	Animal Model	Test Material and Study Duration	Results and Observations	Comments	Reference
			inhibited in the juice and ethanol extracts groups in a dose-dependent manner.		
Anti-amnesic effect	6-week-old male ICR mice	Oral administration of 0, 5, 10, 20, or 40 mg per kg bw ashitaba leaf ethanol extract, 10 mg per kg bw tacrin (anti-amnesia positive control), or 10% Tween 80 solution (vehicle control) one hour prior to task; i.p. injection of 1 mg per kg bw scopolamine 30 minutes later	Ashitaba leaf ethanol extract reduced scopolamine-induced acetylcholinesterase activity and prevented reduction of brain-derived neurotrophic factor expression and camp response element-binding protein expression in the hippocampus.	Ashitaba leaf ethanol extract had a sedative effect on mice. No adverse effects were reported.	Oh et al. (2012)
Eye irritancy	9-week-old male New Zealand white rabbits (3 per group)	Optical drip of 100 mg per mL aqueous or ethanol extracts prepared from dried, pulverized *A. keiskei* leaf	Aqueous and ethanol extracts of ashitaba leaf did not induce perforated ocular lesions, or effect the size or turbidity, of the cornea, induce swelling of the eyelid, and no emissions were observed in the eye or eyelid/eyelashes following treatment.	No adverse effects were reported.	Son et al. (2012)
Skin irritancy	9-week-old male New Zealand white rabbits	Topical application of 10 mg per mL aqueous or ethanol extracts prepared from dried, pulverized *A. keiskei* leaf	Aqueous and ethanol extracts of ashitaba leaf did not affect damaged or unwounded skin in rabbits, and were considered not toxic.	No adverse effects were reported	Lee (2013)
Phototoxicity	7-week-old male Hartley guinea pigs	Topical application of 0.5 mg per mL aqueous or ethanol extracts prepared from dried, pulverized *A. keiskei* leaf	Aqueous and ethanol extracts of ashitaba leaf did not induce skin irritation caused by UV radiation in guinea pigs.	No adverse effects were reported	Lee (2013)
Glucose uptake promotion	Male type 2 diabetic rats	Rats were divided into four groups fed a high-fat diet, and were administered 0, 5, 10, or 30 mg per kw bw ashitaba chalcones.	Significantly higher expression levels of glucose transporter 2 and glucose transporter 4 were observed in the liver and skeletal	Abstract only. Article in Chinese.	Zhao et al. (2013)

Investigation	Animal Model	Test Material and Study Duration	Results and Observations	Comments	Reference
			muscles, respectively, for rats in the high-dose group compared with control. Significantly lower levels of fasting blood glucose and insulin were observed in the high-dose group compared with control.		
Anti-inflammation activity	*Helicobactor pylori*-infected mice	Supplementation with *A. keiskei*	Supplementation with *A. keiskei* suppressed lipid peroxides, myeloperoxidase activity, and inducted of inflammatory meditations, and activation of NF-κB in *H. pylori*-infected mice.	Abstract only.	Kim et al. (2016a)
Obesity-induced inflammatory response	6-week-old male C57BL/6J mice	Oral administration of a high-fat diet supplemented with 0.1% or 0.15% (w/w) XA for 14 weeks	Immunohistological staining for macrophage marker F4/80 showed fewer positive stains in 0.15% XA-treated mice than control; gene expression of inflammatory markers MCP-1 and tumor necrosis factor alpha (TNF-a) was suppressed by XA-treatment.	No adverse effects were reported.	Li et al. (2016)
		Oral administration of 0.15% (w/w) XA for 2 weeks	The anti-inflammatory effect of XA was observed more quickly than the anti-obesity effect.		
Anti-thrombotic effect	7-week-old male Kwl ICR mice	Oral administration of 100 mg lyophilized ashitaba sap (containing 8.7 mg chalcones including 8.7 mg XA and 2.9 mg 4-HD; JBSL, Japan) per mouse per day for seven days; a single peritoneum injection of 100 mg ethyl acetate extract (containing	XA and 4-HD inhibit isolated platelet aggregation; XA is the most potent inhibitor of LPS-induced shortened tail-bleeding in mice.	No adverse effects were reported.	Ohkura et al. (2016)

Investigation	Animal Model	Test Material and Study Duration	Results and Observations	Comments	Reference
		27.1 mg XA and 13.8 mg 4-HD) per kg bw or 10 mg purified chalcones per kg bw was administered and hour before i.p. injection with 0.05 mg per kg lipopolysaccharide (LPS)			
Lipid accumulation inhibition	Zebrafish embryos	Zebrafish embryos were fed twice per day in embryonic water containing standard diet (rotifers and dietary pellets) or high fat cholesterol diet for 10 days; isobavochalcone isolated from *A. keiskei* root was dissolved in DMSO (100 nM) and added to embryonic water for 10 days	Zebrafish embryos fed the high fat cholesterol diet and isobavochalcone did not develop steatosis and a reduced fluorescence level of lipids were observed compared to control	No adverse effects were reported.	Lee et al. (2018)
Anti-tumor activity	6- to 8-week old BRAF V600E/PTEN-null mice with the BRAFV600E mutation heterozygote and PTEN loses with Cre	Topical treatment with 2.5 µL of 1.9 mg per mL 4-hydroxytamoxifen for 3 days, followed by oral administration of 10 or 50 mg per kg bw XA or 4-HD via gavage daily or beginning on study day 23.	Ingestion of 4-HD or XA suppressed the occurrence and development of melanoma, and significantly reduced the tumor weight of already-formed melanomas.	No adverse effects were reported.	Zhang et al. (2018)
Anti-myopathy effect	7-week-old male Sprague-Dawley rats (five groups of six rats each)	Oral administration of 250 or 500 mg per kg bw per day ashitaba root ethanol extract for 28 days; i.p. administration of 1 mg per kg bw dexamethasone for 7 days to induce muscle atrophy	Treatment with 500 mg per kg ashitaba root ethanol extract ameliorated the effects of dexamethasone treatment.	No adverse effects were reported.	Kweon et al. (2019)
Anti-thrombotic effect	Tsumura Suzuki mice	Administration of ashitaba yellow sap	Supplementation with ashitaba yellow sap decreased food efficiency and plasma plasminogen activator inhibitor-1 compared to control. A decrease in plasma glucose, insulin, TNF-α,	Abstract only.	Ohta et al. (2019)

Investigation	Animal Model	Test Material and Study Duration	Results and Observations	Comments	Reference
			and body weight gain, as well as a decrease in liver PAI-1 protein levels, was also observed compared to control.		
Anti-metastatic activity	5-week-old male athymic BALB/c nu/nu SPF mice	Huh7 human liver carcinoma cells were injected into the tail veins of mice (1×10^7 cells per mL in saline). Mice were randomized into three treatment groups of 6 mice each: control or 40 mg per kg XA; or 80 mg per kg XA by i.p. injection. Mice were euthanized after 4 weeks.	Treatment with 40 or 80 mg per kg per day XA ameliorated the extensive inflammatory cell infiltration observed in the lung tissue of the control group in a dose-dependent manner. A reduction of pulmonary metastatic nodules in the treatment groups compared with control was also observed.	No adverse effects were reported.	Yang et al. (2019)
Effectiveness as a feed additive	Nile tilapia fry, mean weight 0.3 g	Oral administration of commercial feed supplemented with 0%, 5%, 10%, and 15% ashitaba leaf powder.	Comparable growth observed in all treatment groups after 45 days. Significant increase in survival rate in the 5% and 10% ashitaba leaf groups compared to control, whereas a significant reduction in survival rate was observed at 15% supplementation.	No adverse effects were reported.	Tattao et al. (2020)
Neuroprotective Effect	Rats with cerebral ischemia reperfusion	XA	Infarct size and brain edema shrank in middle cerebral artery occlusion upon treatment with XA.	Abstract only.	Chao et al. (2021)

TPA – 12-*O*-tetradecanoylphorbol-13-acetate; DMBA – 7,12-dimethylbenz[*a*]anthracene; 4-HD – 4-hydroxyderricin; XA –xanthoangelol; i.p. – intraperitoneal; LDL –low-density lipoprotein; VLDL –very-low density lipoprotein; HDL –high-density lipoprotein; GalN –D-galactosamine; CCl_4 –carbon tetrachloride; PAI-1 –plasminogen activator inhibitor-1; DNFB –1-Fluoro-2,4-dinitrobenzene; NF-KB –nuclear factor -κB; TNF-a –tumor necrosis factor alpha; LPS –lipopolysaccharide; DMSO –dimethyl sulfoxide

Appendix 9. Clinical Studies with Ashitaba-Containing Test Articles

Appendix 9. Table 1. Human clinical studies on *A. keiskei*-containing green juice

Investigation	Subjects	Test Material and Study Duration	Results and Observations	Comments	Reference
Effect on peripheral lymphocytes DNA damage	20 male smokers (mean age = 36.3 ± 2.2 years) with no previously diagnosed medical conditions and an average cigarette consumption of 16.2 ± 2.6 packs per year.	Subjects were given 240 mL of commercially available Korean green vegetable drink containing *A. keiskei* aqueous extract (amount unknown), kale extract, turmeric, and rooibos tea every day for 8 weeks. Subjects were asked to continue their normal level of smoking, and to restrict their intake of red, yellow, green and orange vegetables and fruits.	A significant reduction in lymphocyte DNA damage was observed at the conclusion of the study, including significant reduction in tail length, tail moment, and percent DNA in the Comet assay.	No adverse effects were reported. Test material was a mixture; therefore, it is impossible to attribute the observations to *A. keiskei* extract alone.	Kang et al. (2004)
Effect on hypercholesterolemic adults	35 Korean hypercholesterolemic adults with ≥200 mg per dL serum total cholesterol or ≥130 mg per dL LDL-cholesterol	Subjects received an *A. keiskei* and turmeric extract (14 females and 7 males) or placebo (control, 11 females and 3 males) for 4 weeks.	There were no changes in serum total cholesterol, LDL-cholesterol, or HDL-cholesterol between the groups. In treatment group subjects, the LDL: HDL ratio and serum prostaglandin E_2 was significantly decreased. No changes were observed in IL-1 β, IL-6, IL-8, 8-isoprostane, malondialdehyde, total antioxidant capacity, and oxidized-LDL levels.	Test material was a mixture; therefore, it is impossible to attribute the observations to *A. keiskei* extract alone. Article in Korean.	Yun et al. (2009)
Effect on postprandial blood glucose levels	Men and women aged 20 years or older with normal glucose	Single dose of commercially available mixed vegetable juice, "Kagome Yasai-ichinichi-	Ingestion of *A. keiskei*-containing vegetable juice with or prior-to a carbohydrate-based meal	Test material was a mixture; therefore, it is	Kasuya et al. (2016)

Investigation	Subjects	Test Material and Study Duration	Results and Observations	Comments	Reference
	tolerance during medical check-ups for the previous year and who were not receiving drug therapy.	koreippon" (Kagome Co., Ltd., Japan) containing various vegetables including and unknown amount of *A. keiskei*.	attenuated the elevation of post prandial blood glucose levels.	impossible to attribute the observations to *A. keiskei* extract alone.	
Effect on alcohol hangovers	15 healthy adults (11 men, 4 women, 29.3±7.0 years old)	In a randomized, double-blinded crossover study, subjects were asked to abstain from alcohol for 3 days and fast for 12 hours prior to the study. Juice was prepared from *A. keiskei*, green grape, and pear (1:1:1) using a low-speed masticating juicer. Subjects drank either 240 mL water (control) or juice 30 minutes prior to alcohol consumption (1.25 g per kg weight) plus 100 g silken soy curd followed by water or juice.	Consumption of *A. keiskei*-containing juice reduced the increase of alcohol levels in plasma and expiratory-air following alcohol consumption.	Test material was a mixture; therefore, it is impossible to attribute the observations to *A. keiskei* alone.	Kim et al. (2018)

LDL –low-density lipoprotein; HDL –high-density lipoprotein

Appendix 10. *In vitro* Biological Activity Studies

Appendix 10. Table 1. Biological activity studies conducted on *A. keiskei*-derived test articles *in vitro*

Investigation	Test Article	Comments	Reference
Inhibition of gastric H^+, (K^+)-ATPase	XA and 4-HD isolated from *A. keiskei* roots	XA and 4-HD inhibits pig gastric H^+ and $K(^+)$-ATPase in a dose-dependent manner	Murakami et al. (1990)
Antibacterial activity	XA and 4-HD isolated from *A. keiskei* roots	XA and 4-HD exhibit antibacterial activities against gram-positive pathogenic bacteria, but not gram-negative bacteria	Inamori et al. (1991)
Anti-tumor activity	9 coumarins and 2 chalcones isolated from *A. keiskei* roots	XA and 4-HD interacted with the Ca^{2+}-calmodulin complex, which may result in anti-tumor promotion activity	Okuyama et al. (1991)
Inhibition of arachidonic acid metabolism in platelets	Xanthoangelol E isolated from *A. keiskei* roots	Xanthoangelol E inhibits the cyclo-oxygenase and lipoxygenase pathways in platelets	Fujita et al. (1992)
Antibacterial activity	XA and 4-HD isolated from *A. keiskei* yellow pigment	XA and 4-HD exhibit antibacterial activities against gram-positive pathogenic bacteria	Baba et al. (1998) (Article in Japanese)
Inhibition of histamine release	6 chalcone isolated from *A. keiskei* roots	Xanthoangelol B, C, and E inhibited histamine release, XA and 4-HD enhanced histamine release, and xanthoangelol had no effect on histamine release in compound 48/80 treated rat peritoneal mast cells	Nakata and Baba (2001) (Article in Japanese)
Artery relaxation	6 chalcone isolated from *A. keiskei* roots	XA, 4-HD, and xanthoangelols B, E, and F inhibit phenylephrine-induced vasoconstriction	Matsuura et al. (2001)

Investigation	Test Article	Comments	Reference
Antitumor activity	7 coumarins, 5 chalcones, 3 flavanones, and 1 diacetylene isolated from *A. keiskei* sap	XA, 4-HD, xanthoangelols F and H, isobavachalcone, laserpitin, isolaserpitin, 3′-senecioyl khellactone, 4′-senecioyl khellactone, pteryxin, and (3′R)-hydroxycolumbianadin are anti-tumor promoters	Akihisa et al. (2003)
Antioxidant activity	6 antioxidative compounds isolated from *A. keiskei* aerial parts	Luteolin 7-*O*-β-D-glucopyranoside, quercetin 3-*O*-β-D-galactopyranoside, quercetin 3-*O*-β-D-glucopyranoside, quercetin 3-*O*-α-D-arabinopyranoside, kaempferol 3-*O*-α-D-arabinopyranoside, and luteolin 7-*O*-rutinoside exhibited DPPH-radical-scavenging activity.	Kim et al. (2005)
Antioxidant activity	Isoquercetin and hyperoside isolated from *A. keiskei* (plant part unknown)	Isoquercetin and hyperoside inhibited DPPH radical, ABTS radical, OH radical, and H_2O_2, and H_2O_2-induced oxidative DNA damage in human lymphocyte cells, in a dose-dependent manner	Shim et al. (2005) (Article in Korean)
Suppression of NF-κB	Xanthoangelols D, E, F, and XA isolated from *A. keiskei* roots	Only xanthoangelol D suppressed basal and TNF-α-induced endothelin-1 production by inhibiting NF-κB activation in porcine aortic endothelial cells	Sugii et al. (2005)
Apoptosis in neuroblastoma and leukemia cells	XA isolated from *A. keiskei* sap	XA induces caspase-3-dependent apoptotic cell death in human neuroblastoma (IMR-32) and leukemia (Jurkat) cells	Tabata et al. (2005)
Antitumor activity	3 coumarins, 6 chalcones, 3 flavanones, and 1 diacetylene isolate from *A. keiskei* sap	Xanthoangelols I and J, isobavachalcone, osthenol, mundulea flavanone B, and 8-geranylnaringenin inhibited chemical carcinogenesis	Akihisa et al. (2006)

Investigation	Test Article	Comments	Reference
Apoptosis in neuroblastoma	6 chalcones isolated from *A. keiskei* sap	All chalcones exhibited cytotoxicity against neuroblastoma cells; isobavachalcone and xanthoangelol H had no effect on normal cells	Nishimura et al. (2007)
Apoptosis in neuroblastoma	XA isolated from *A. keiskei* sap	XA induces mitochondria-mediated apoptosis in neuroblastoma cells	Motani et al. (2008)
Superoxide-scavenging	Xanthokeismins A, B, and C, and Xanthoangelol B isolated from *A. keiskei* stems	Xanthokeismins A, B, and C, and Xanthoangelol B exhibit superoxide-scavenging activity with IC_{50} values ranging from 0.51 to 1.1 μM	Aoki et al. (2008)
Apoptosis in stomach cancer cells	XA and 4-HD isolated from *A. keiskei* stems	XA and 4-HD induces apoptosis in stomach cancer cells	Takaoka et al. (2008)
Superoxide-scavenging	Luteolin, protocatechuic acid, guijaverin, hyperoside, and cymaroside isolated from *A. keiskei* leaves	Luteolin and protocatechuic acid showed DPPH radical scavenging activity.	Jo and Park (2008) (Article in Korean)
Anti-allergenic agent	Selinidin isolated from *A. keiskei* sap	Selinidin suppresses immunoglobulin E-mediated mast cell activation	Kishiro et al. (2008)
Antitumor activity	9 chalcones, 5 coumarins, 4 flavanones isolated from *A. keiskei* roots	4-HD exhibited potent cytotoxic activity against human tumor cell lines HL60 (leukemia), CRL1579 (melanoma), A549 (lung), and AZ521 (stomach)	Akihisa et al. (2011)
Anti-inflammation activity	6 chalcones isolated from *A. keiskei* leaves	2′,4′,4-trihydroxy-3′-[2-hydroxy-7-methyl-3-methylene-6-octaenyl]chalcone, 2′,4′,4-trihydroxy-3′-geranylchalcone, and 2′,4′,4-trihydroxy-3′-[6-hydroxy-3,7-dimethyl-2,7-octadienyl]chalcone inhibit IL-6 production in TNF-α-stimulated MG-63 cells.	Shin et al. (2011) (Abstract only)
Metabolic syndrome prevention	6 chalcones isolated from *A. keiskei* roots	Ashitaba root chalcones induce the production of adiponectin in 3T3-L1 adipocytes	Ohnogi et al. (2012e)

Investigation	Test Article	Comments	Reference
Melanin biosynthesis inhibition	4-HD, XA, xanthoangelol H, and deoxyxanthoangelol H isolated from *A. keiskei* stem	4-HD, XA, xanthoangelol H, and deoxyxanthoangelol H inhibited melanin formation in B16 melanoma cells, with IC_{50} values ranging from 11.6 to 21.4 μM, with low observed cytotoxicity.	Arung et al. (2012)
Monoamine oxidase inhibition	XA, 4-HD, and cymaroside isolated from *A. keiskei* aerial parts	XA non-selectively inhibits monoamine oxidase (MAO), with IC_{50} values of 43.4 μM and 43.9 μM for MAO-A and MAO-B, respectively. XA inhibited dopamine β-hydroxylase with an IC_{50} value of 0.52 μM. 4-HD selectively inhibits MAO-B (IC_{50} value of 3.43 μM) and mildly inhibits dopamine β-hydroxylase (DBH) activity. Cynaroside is a potent DBH inhibitor, with and IC_{50} value of 0.0410 μM.	Kim et al. (2013)
Adipocyte differentiation inhibition	4-HD and XA isolated from Ashitaba Chalcone Powder (JBSL, Japan)	4-HD and XA inhibit adipocyte differentiation by downregulating the expression of adipocyte-specific transcription factors in 3T3-L1 adipocytes	Zhang et al. (2013)
Anti-inflammation activity	7 chalcones isolate from *A. keiskei* aerial parts	4-HD, xanthoangelols B and E, and xanthokeismin A inhibited nitric oxide (NO) production and the expression of IL-1β and IL-6 in LPS-activated microphages, suppressed the degradation of inhibitory-κBα, and suppressed the translocation of NF-κB into the nuclei of LPS-activated microphages.	Chang et al. (2014)
Xanthine oxidase inhibition	16 metabolites isolated from *A. keiskei* root	4-HD, XA, isobavachalcone, and	Kim et al. (2014)

Investigation	Test Article	Comments	Reference
	bark, stems, leaves, and root cores	xanthoangelols B and F exhibited mixed inhibition of xanthine oxidase, with IC_{50} values ranging from 8.1 to 54.3 μM	
Anti-inflammation activity	4-HD and XA isolated from ashitaba chalcone powder (JBSL, Japan)	4-HD and XA reduced lipopolysaccharide-induced production of nitric oxide, secretion of tumor necrosis factor-alpha, and expression of inducible NO synthase and cyclooxygenase-2; 4-HD and XA reduced phosphorylation of the p65 subunit of NF-κB	Yasuda et al. (2014)
Heat shock protein inducing activity	13 chalcones isolated from *A. keiskei* aerial parts	(±)-4,2′-4′-trihydroxy-3′-[(6*E*)-2-hydroxy-7-methyl-3-methylene-6-octenyl]chalcone activated the *hsp25* promoter , and increased expression of HSF1, HSP70, and HSP27, without any significant cellular cytotoxicities. 4,2′-4′-trihydroxy-3′-[(2*E*,5*E*)-7-methoxy-3,7-dimethyl-2,5-octadienyl]chalcone also activated the *hsp25* promoter.	Kil et al. (2015)
Anti-thrombotic effect	4-HD, XA, and xanthoangelols B, D, E, and F (source not reported)	XA suppressed TNF-α-induced PAI-1 increased in a dose-dependent manner, and was cytotoxic to EA.hy926 cells at 25 μM; none of the other test articles inhibited TNF-α-induced PAI-1 increase, though xanthoangelol was significantly cytotoxic to EA.hy926 cells at 10 μM	Ohkura et al. (2015)
Anti-inflammation activity	4-HD and XA isolated from *A. keiskei* roots	4-HD and XA moderated the suppression of uncoupling protein 1 promotor activity and gene expression in C3H10T1/2 adipocytes, and inhibited c-Jun N-terminal kinase	Li et al. (2016)

Investigation	Test Article	Comments	Reference
		phosphorylation, NF-κB, and activator protein 1.	
Inhibition of viral proteases	9 chalcones and 4 coumarins isolated from *A. keiskei* leaves	Xanthoangelol E exhibited potent chymotrypsin-like protease and papain-like protease activities against severe acute respiratory syndrome coronavirus, with IC_{50} values of 11.4 and 1.2 μM, respectively.	Park et al. (2016)
Anti-hepatotoxic effect	Ethanol extract of dried *A. keiskei* (plant part unknown)	Ethanolic extract of *A. keiskei* prevented acetaminophen-induced hepatotoxicity in HepG2 human hepatocellular liver carcinoma and HepaRG human hepatic progenitor cells by increasing cell grown and decreasing lactate dehydrogenase leakage in a dose-dependent manner. Ethanolic extract of *A. keiskei* also modulates the expression of Bcl-2 family proteins and decreases the cleavage level of caspase-9, -7, and-3 and PARP under acetaminophen-induced hepatotoxicity.	Choi et al. (2017)
Cell proliferation effects	8 compounds isolated from *A. keiskei* aerial parts	(-)-*cis*-(3′R,4′R)-4′-*O*-β-D-glucopyranoside, 3′-[(2E)-5-carboxy-3-methyl-2-pentenyl]-4,2′,4′-trihydroxychalcone, (±)-4,2′,4′-trihydroxy-3′-[2-hydroxy-2-[tetrahydro-2-methyl-5-(1-methylethenyl)-2-furanyl]ethyl]chalcone, (R)-*O*-isobutyroyllomatin, 3′-*O*-methylvaginol, (-)-jejuchalcone F, isoliquiritigenin, davidigenin, and (±)-liquiritigenin exhibited cell proliferative effects on Hep3B human hepatoma	Kil et al. (2017) (Abstract only)

Investigation	Test Article	Comments	Reference
		cells and showed dose-dependent protection against oxidative stress.	
Antituberculosis activity	Ethanol extract of freshly dried *A. keiskei* stem	Ethanolic extract of *A. keiskei* stem displayed a minimum inhibitory effect from 6% to 8% (w/v) on *Mycobacterium tuberculosis* strain H37Rv.	Kusuma et al. (2018)
Dipeptidyl peptidase-IV inhibition	4-HD isolated from *A. keiskei* sap	4-HD inhibits dipeptidyl peptidase-IV with an IC_{50} value of 81.44 µM.	Aulifa et al. (2019)
Effect on osteoclasts and osteoblasts	4-HD isolated from *A. keiskei* chalcone-rich powder (JBSL, Japan)	4-HD inhibited formation of multinucleated osteoclasts in culture and induced osteoblastic differentiation.	Hagiwara et al. (2019)
Anti-metastatic activity	Synthesized XA	XA suppressed human hepatocellular carcinoma cell migration, invasion, and epithelial-mesenchymal transition, as well as induced autophagy by activating the AMPK/mTOR signaling pathway.	Yang et al. (2019)

4-HD – 4-hydroxyderricin; XA –xanthoangelol; DPPH –1,1-diphenyl-2-picryl-hydrazl; ABTS –2,2′-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid); OH –hydroxide; IC_{50} –half maximal inhibitory concentration; MAO –monoamine oxidase; DBH –dopamine β-hydroxylase; NO –nitric oxide; LPS –lipopolysaccharide; TNF-a –tumor necrosis factor alpha; PAI-1 –plasminogen activator inhibitor-1

END

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

GENERALLY RECOGNIZED AS SAFE (GRAS) NOTICE

Form Approved: OMB No. ; Expiration Date:
(See last page for OMB Statement)

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
001012	07/06/2021
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

RECEIVED
JUL 06 2021
OFFICE OF FOOD ADDITIVE SAFETY

Transmit completed form and attachments electronically via the Electronic Submission Gateway *(see instructions)*; OR Transmit completed form and attachments in paper format or on physical media to: Office of Food Additive Safety *(HFS-200)*, Center for Food Safety and Applied Nutrition, Food and Drug Administration, 5100 Paint Branch Pkwy., College Park, MD 20740-3835.

PART I – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*

☒ New ☐ Amendment to GRN No. _____ ☐ Supplement to GRN No. _____

2. ☒ All electronic files included in this submission have been checked and found to be virus free. *(Check box to verify)*

3a. For New Submissions Only: Most recent presubmission meeting *(if any)* with FDA on the subject substance *(yyyy/mm/dd)*: N/A

3b. For Amendments or Supplements: Is your amendment or supplement submitted in response to a communication from FDA? *(Check one)* ☐ Yes ☐ No If yes, enter the date of communication *(yyyy/mm/dd)*: _____

PART II – INFORMATION ABOUT THE NOTIFIER

1a. Notifier

Name of Contact Person	Position
Vincent Hackel	President/CEO

Company *(if applicable)*: Japan Bio Science Laboratory-USA, Incorporated

Mailing Address *(number and street)*: 1547 Palos Verdes Mall, #131

City	State or Province	Zip Code/Postal Code	Country
Walnut Creek	California	94597	United States of America

Telephone Number	Fax Number	E-Mail Address
925-938-2732	925-407-2994	v_hackel@jbsl-net.com

1b. Agent or Attorney *(if applicable)*

Name of Contact Person	Position
Katrina Emmel	President

Company *(if applicable)*: KemmelCal Inc.

Mailing Address *(number and street)*: 947 Martina Circle

City	State or Province	Zip Code/Postal Code	Country
Corona	California	92879	United States of America

Telephone Number	Fax Number	E-Mail Address
847-436-2598	N/A	katrina.emmel@kemmelcal.com

PART III – GENERAL ADMINISTRATIVE INFORMATION

1. Name of Substance

Ashitaba Chalcone Powder (8%)

2. Submission Format: *(Check appropriate box(es))*

☐ Electronic Submission Gateway

☐ Paper

☒ Electronic files on physical media with paper signature page

If applicable give number and type of physical media

3. For paper submissions only:

Number of volumes _____

Total number of pages _____

4. Does this submission incorporate any information in FDA's files by reference? *(Check one)*

☐ Yes *(Proceed to Item 5)* ☒ No *(Proceed to Item 6)*

5. The submission incorporates by reference information from a previous submission to FDA as indicated below *(Check all that apply)*

☐ a) GRAS Notice No. GRN _____

☐ b) GRAS Affirmation Petition No. GRP _____

☐ c) Food Additive Petition No. FAP _____

☐ d) Food Master File No. FMF _____

☐ e) Other or Additional *(describe or enter information as above)* _____

6. Statutory basis for determination of GRAS status *(Check one)*

☒ Scientific Procedures *(21 CFR 170.30(b))* ☐ Experience based on common use in food *(21 CFR 170.30(c))*

7. Does the submission (including information that you are incorporating by reference) contain information that you view as trade secret or as confidential commercial or financial information?

☐ Yes *(Proceed to Item 8)*

☒ No *(Proceed to Part IV)*

8. Have you designated information in your submission that you view as trade secret or as confidential commercial or financial information *(Check all that apply)*

☐ Yes, see attached Designation of Confidential Information

☐ Yes, information is designated at the place where it occurs in the submission

☐ No

9. Have you attached a redacted copy of some or all of the submission? *(Check one)*

☐ Yes, a redacted copy of the complete submission

☐ Yes, a redacted copy of part(s) of the submission

☐ No

PART IV – INTENDED USE

1. Describe the intended use of the notified substance including the foods in which the substance will be used, the levels of use in such foods, the purpose for which the substance will be used, and any special population that will consume the substance *(e.g., when a substance would be an ingredient in infant formula, identify infants as a special population)*.

JBSL-USA intends to use Ashitaba Chalcone Powder (8%) as an ingredient in conventional foods for the general population in: soft drinks (up to 0.0075%); jelly, jams, preserves, and marmalade (up to 0.834%); candy containing chocolate (up to 0.006%); butter (up to 0.468%); and at a maximum use level of 125 mg per day.

2. Does the intended use of the notified substance include any use in meat, meat food product, poultry product, or egg product? *(Check one)*

☐ Yes ☒ No

PART V – IDENTITY

1. Information about the Identity of the Substance

	Name of Substance[1]	Registry Used *(CAS, EC)*	Registry No.[2]	Biological Source *(if applicable)*	Substance Category *(FOR FDA USE ONLY)*
1	Ashitaba Chalcone Powder	N/A	N/A	*Angelica keiskei*	
2					
3					

[1] Include chemical name or common name. Put synonyms *(whether chemical name, other scientific name, or common name)* for each respective item (1 - 3) in Item 3 of Part V *(synonyms)*

[2] Registry used e.g., CAS *(Chemical Abstracts Service)* and EC *(Refers to Enzyme Commission of the International Union of Biochemistry (IUB), now carried out by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUBMB))*

2. Description

Provide additional information to identify the notified substance*(s)*, which may include chemical formula*(s)*, empirical formula*(s)*, structural formula*(s)*, quantitative composition, characteristic properties *(such as molecular weight(s))*, and general composition of the substance. For substances from biological sources, you should include scientific information sufficient to identify the source *(e.g., genus, species, variety, strain, part of a plant source (such as roots or leaves), and organ or tissue of an animal source)*, and include any known toxicants that could be in the source.

Ashitaba Chalcone Powder (8%) is prepared from the sap of *Angelica keiskei* (Miq.) Koidz. (syn. *Archangelica keikei* Miq.). JBSL-USA's Ashitaba Chalcone Powder (8%) preparation is a yellow powder primarily composed of carbohydrates, fat, chalcones (predominantly 4-hydroxyderricin and xanthoangelol), and moisture. There are no known toxicants.

3. Synonyms

Provide as available or relevant:

1	Ashitaba or asitaba
2	Ashitaba chalcone sap powder
3	ChalCurb-P8

PART VI – OTHER ELEMENTS IN YOUR GRAS NOTICE

(check list to help ensure your submission is complete – check all that apply)

- ☒ Any additional information about identity not covered in Part V of this form
- ☒ Method of Manufacture
- ☒ Specifications for food-grade material
- ☒ Information about dietary exposure
- ☒ Information about any self-limiting levels of use *(which may include a statement that the intended use of the notified substance is not-self-limiting)*
- ☒ Use in food before 1958 *(which may include a statement that there is no information about use of the notified substance in food prior to 1958)*
- ☒ Comprehensive discussion of the basis for the determination of GRAS status
- ☒ Bibliography

Other Information

Did you include any other information that you want FDA to consider in evaluating your GRAS notice?

☒ Yes ☐ No

Did you include this other information in the list of attachments?

☒ Yes ☐ No

PART VII – SIGNATURE

1. The undersigned is informing FDA that Japan Bio Science Laboratory-USA *(name of notifier)*

has concluded that the intended use(s) of Ashitaba Chalcone Powder (8%) *(name of notified substance)*

described on this form, as discussed in the attached notice, is (are) exempt from the premarket approval requirements of section 409 of the Federal Food, Drug, and Cosmetic Act because the intended use(s) is (are) generally recognized as safe.

2. ☒ Japan Bio Science Laboratory-USA *(name of notifier)* agrees to make the data and information that are the basis for the determination of GRAS status available to FDA if FDA asks to see them.

Japan Bio Science Laboratory-USA *(name of notifier)* agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so.

1547 Palos Verdes Mall, #131, Walnut Creek, CA 94597 *(address of notifier or other location)*

Japan Bio Science Laboratory-USA *(name of notifier)* agrees to send these data and information to FDA if FDA asks to do so.

OR

☐ The complete record that supports the determination of GRAS status is available to FDA in the submitted notice and in GRP No. _____ *(GRAS Affirmation Petition No.)*

3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
Katrina Emmel Digitally signed by Katrina Emmel Date: 2021.06.25 20:22:01 -07'00'	Katrina Emmel, President	06/25/2021

PART VIII – LIST OF ATTACHMENTS

List your attached files or documents containing your submission, forms, amendments or supplements, and other pertinent information. Clearly identify the attachment with appropriate descriptive file names (or titles for paper documents), preferably as suggested in the guidance associated with this form. Number your attachments consecutively. When submitting paper documents, enter the inclusive page numbers of each portion of the document below.

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Appendices 1 through 10	